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Special Issue Article

Phylogenomic analysis reveals a two-stage process of the evolutionary transition of *Shewanella* from the upper ocean to the hadal zone

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Summary

Shewanella **strains are characterized by versatile metabolic capabilities, resulting in their wide distribution in the ocean at different depths. Considering that particle sedimentation is an important dynamic process in the ocean, we hypothesized that hadal *Shewanella* species evolved from the upper ocean. In this study, we isolated three novel *Shewanella* strains from deep-sea sediments in the Southwest Indian Ocean. Genome sequencing indicated that strains YLB-06 and YLB-08 represent two novel species in the genus *Shewanella*. Through phylogenomic analysis, we showed that speciation and genomic changes in marine *Shewanella* strains are related to water depth. We further confirmed the aforementioned hypothesis and revealed a two-stage process of the evolutionary transition of *Shewanella* from the upper ocean to the hadal zone by comparative genomics and gene gain/loss analysis. Finally, the transcriptomic analysis demonstrated that recently obtained genes are strictly repressed and may thus play a minor role in the response to environmental changes.**

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Introduction

The ocean is the largest ecosystem on the planet, and it contains a large variety of microorganisms (Sunagawa *et al*., 2015; Ibarbalz *et al*., 2019). Different marine areas have very distinct environmental characteristics, and microorganisms occupy different niches by means of diverse metabolic capabilities and survival strategies (Orcutt *et al*., 2011). The microbial genome provides an important data source for analysing environmental adaptation mechanisms and species formation processes (Lawrence and Hendrickson, 2005; Abby and Daubin, 2007). Multiple hypotheses have been used to explain genome changes in prokaryotes. Among these hypotheses, the genome streamlining theory, which suggests that there are survival and reproductive benefits to microorganisms possessing a smaller genome size with fewer non-essential genes and less non-coding DNA, has been used to explain the cosmopolitan marine bacterial SAR11 clade (Giovannoni *et al*., 2005; Giovannoni *et al*., 2014). Recently, this theory has been verified in the habitat transition from freshwater sediment to a pelagic existence in the family Methylophilaceae (Salcher *et al*., 2019). In addition, a new hypothesis called 'trophic specialization' was recently proposed based on comparative genomic and physiological studies of the genera *Idiomarina* and *Kangiella* (Qin *et al*., 2019).

At a water depth >6000 m, the hadal zone is the deepest area in the ocean and represents one of the least-studied environments on the planet. Although it covers only 1%–2% of the global deep ocean floor, it accounts for up to 45% of the vertical depth of the ocean (Blankenship-Williams and Levin, 2009; Jamieson *et al*., 2010). Previously, the hadal zone was considered to be an area where life is not possible. However, recent studies have shown that the hadal ecosystem has a unique microbial community structure and actively participates in biogeochemical processes (Ichino *et al*., 2015; Jamieson, 2015). As part of the marine environment, the hadal biosphere exhibits a wide range of material and energy exchanges with the upper oceans, which together constitute the total organic component of the ocean and even the global ecosystem (Jamieson *et al*., 2010). The hadal zone is characterized by multiple extreme environmental conditions, such as high hydrostatic pressure, low temperature, darkness and deficiency of biodegradable nutrients (Jamieson, 2001; Jamieson *et al*., 2010). Previous studies have shown that the distribution, composition and metabolic potential of hadal microbes are rather special (Nunoura *et al*., 2015; Liu *et al*., 2019; Wang *et al*., 2019), and some novel bacterial species, such as *Moritella yayanosii* DB21MT-5 (Nogi and Kato, 1999), *Psychrobacter pacificensis* P2K6 (Maruyama *et al*., 2000), *Colwellia piezophila* Y223G (Nogi *et al*., 2004), *C. marinimaniae* MTCD1 (Kusube *et al*., 2017), *Rhodobacterales* bacterium PRT1 (Eloe *et al*., 2011), *Profundimonas piezophile* YC-1, *Corynebacterium hadale* NBT06-6 (Wei *et al*., 2018), *Bacillus piezotolerans* YLB-04 (Yu *et al*., 2019b) and *Marinomonas piezotolerans* YLB-05 (Yu *et al*., 2019a), have been isolated. Nevertheless, the speciation and evolution of hadal microorganisms remain largely unexplored.

The *Shewanella* genus is widely distributed in a variety of environments, especially marine and deep-sea sediments, due to its remarkable ability to utilize multiple electron receptors and its versatile metabolic capabilities (Hau and Gralnick, 2007; Fredrickson *et al*., 2008). *Shewanella* species have been isolated from diverse marine environments, and many aspects of their characteristics have been explored (Nogi *et al*., 1998; Toffin *et al*., 2004; Wang *et al*., 2004; Gao *et al*., 2006). Two *Shewanella benthica* strains, KT99 and DB21MT-2, possessing a relatively small genome (4.35 Mb) were isolated from the Kermadec and Mariana Trenches at depths of 9856 m and 10 898 m respectively (Lauro *et al*., 2013; Zhang *et al*., 2019). Additionally, a psychrophilic and piezophilic *Shewanella* strain, *S. violacea* DSS12, was isolated from abyssopelagic sediment in the Ryukyu Trench at a depth of 5110 m (Aono *et al*., 2010). Since *Shewanella* strains have been frequently isolated in oceans at different depths and have been shown to be one of the dominant microbial groups in abyssal sinking particles (Boeuf *et al*., 2019), we hypothesized that there was an evolutionary process corresponding to the transition of *Shewanella* from the upper ocean to the hadal zone. In this study, we isolated three *Shewanella* strains from deep-sea sediments in the Southwest Indian Ocean. Through comparative genomics, the investigation of gene gains and losses, and transcriptome analysis, we confirmed the aforementioned hypothesis and revealed the evolutionary trajectory of marine *Shewanella*.

Results and discussion

Isolation of deep-sea bacteria with the largest genome within Shewanella *genus*

Based on the aforementioned hypothesis, which was derived from the observation that *Shewanella* species are widely distributed in the ocean at different water depths, we speculated that *Shewanella* strains evolved from the shallow sea to inhabit the deep ocean and then to the hadal zone. Therefore, better elucidation of this evolutionary process will require more sequence data from deep-sea *Shewanella* species, which are in a key intermediate transition state. Initially, we isolated and identified several deep-sea bacteria from two sediment samples collected at water depths of 2315 and 2699 m in the Southwest Indian Ocean. The results of 16S rRNA gene amplification and DNA sequence comparison identified three strains belonging to the *Shewanella* genus, designated *Shewanella* sp. YLB-06, *Shewanella* sp. YLB-08 and *Shewanella* sp. YLB-09. Among these strains, *Shewanella* sp. YLB-06 shared the highest similarity (98.43%) of the 16S rRNA gene with *S. benthica* ATCC 43992, while the sequences of *Shewanella* sp. YLB-08 and *Shewanella* sp. YLB-09 most closely resembled *S. sediminis* HAW-EB3, with identities of 98.3% and 98.4% respectively.

Next, the whole genomes of the three *Shewanella* strains were sequenced, and the genome sizes of YLB-06, YLB-08 and YLB-09 were 6.45, 5.78 and 6.23 Mb respectively (Fig. S1), which exceed the genome sizes of the majority of *Shewanella* species. In particular, YLB-06 has the largest genome among the reported genomes of the *Shewanella* genus. The chromosomal DNA G + C contents of the three strains were 45.1, 43.6 and 43.5 mol% (Table S1) respectively, which are lower than those of the *Benthica* clade of *Shewanella* species (47–49 mol%), as reported previously (Fang *et al*., 2019). Moreover, the DNA–DNA hybridization (DDH) and average nucleotide identity (ANI) estimates between strains YLB-06 and YLB-08 and their closest type strains were significantly lower than the proposed cutoff level (70% and 95%–96%) for species delineation (Stackebrandt

et al., 2002; Richter and Rosselló-Móra, 2009), suggesting that strains YLB-06 and YLB-08 may represent two novel species in the genus *Shewanella* (Table S2).

Speciation and genomic changes in marine Shewanella *strains are related to water depth*

To determine the evolutionary status of these three newly isolated strains, we selected 41 *Shewanella* strains with complete genomes that were publicly available prior to 8 June 2019 and constructed a phylogenetic tree based on 73 conserved marker genes by using the maximum likelihood method. The results showed that the strains from the marine environment constituted a large clade of the *Shewanella* genus. The deep-sea *Shewanella* bacteria isolated to date belonged to three different branches, and the three strains obtained in this study belonged to the largest branch among them (Fig. 1A and Fig. S2).

According to the phylogenetic tree, *Shewanella* sp. YLB-06 was located between *S*. *psychrophila* WP2 and *S*. *violacea* DSS12, which were isolated from water depths of 1914 and 5110 m respectively (Xiao *et al*., 2007; Aono *et al*., 2010) (Fig. 1B), indicating that it is a species undergoing a transition from the deep ocean to the abyssal zone. *Shewanella* sp. YLB-08 and *Shewanella* sp. YLB-09 belonged to the same independent branch located between *S*. *sediminis* HAW-EB3 (isolated at a water depth of 215 m) and *S*. *psychrophila* WP2 (Fig. 1B), suggesting that they are early species that evolved from shallow water to inhabit the deep sea. The phylogenetic tree further revealed that the marine *Shewanella* clade contained three main clusters, all of which included both shallow and deep-sea strains, indicating that there are multiple evolutionary branches of benthic *Shewanella*. The third cluster contained two abyssal bacteria (Fig. 1B), thus providing an exceptional model for tracing *Shewanella* evolution from the surface ocean to the deepest layer.

Notably, the genome size of marine *Shewanella* strains was significantly related to water depth. The deep-sea *Shewanella* species generally exhibited a larger genome and gene capacity than their shallow-sea relatives (Fig. 1C). Interestingly, we noted that the evolution of *Shewanella* from the deep sea to the abyss was associated with a significant genome reduction. *Shewanella benthica* DB21MT-2, which was isolated from the bottom of the Mariana trench, exhibited a genome size close to the smallest genomes among the *Shewanella* genus (Thorell *et al*., 2019; Zhang *et al*., 2019). Nevertheless, there was no significant pattern in terms of the changes in the GC contents of the genomes, except that the first cluster possessed a higher GC content than the other two clusters (Fig. 1C). Additionally, the carbon contents of the encoded proteins (C-ARSC) and the numbers of nitrogen atoms per residue side chain (N-ARSC) in these strains were calculated, and no significant difference was observed (Supporting Information Fig. S3), indicating that the demand for carbon and nitrogen sources has played a minor role in the speciation of marine *Shewanella* strains.

According to the functional classification of gene ontology (GO) and clusters of orthologous groups (COG), we analysed the differences in the functional gene composition between different *Shewanella* strains. The results showed that the strains within a given clade exhibited similar compositions, but there was no significant difference in the functional gene composition between epi/mesopelagic and bathypelagic *Shewanella* strains (Fig. 2A and Fig. S4). For the abysso/hadalpelagic *Shewanella* strains, a higher proportion of genes related to COG-J (translation, ribosomal structure and biogenesis) and lower proportions of genes related to COG-T (signal transduction mechanisms) and COG-K (transcription) were observed and compared with the bathypelagic species (Fig. 2A), suggesting that abysso/hadalpelagic bacteria exhibit a higher demand for genes involved in protein synthesis and a reduced need for genes related to RNA transcription and signal transduction. In addition, *S*. *benthica* DB21MT-2 showed some differences compared with *S*. *violacea* DSS12 in terms of the COG composition (Fig. 2), suggesting that it has developed unique genomic features at the bottom of the Mariana Trench.

It is worth noting that there was a significant difference in functional gene composition between different deep-sea *Shewanella* species. For example, *S*. *psychrophila* WP2 and *S*. *piezotolerans* WP3 differed significantly in the relative abundance of genes belonging to COG-J, COG-T, COG-C (energy production and conversion) and COG-X (mobilome: prophages, transposons) (Fig. 2B), although they were isolated from the same geographic site (Wang *et al*., 2004). This result indicates that different strains may have adopted different strategies to adapt to the deep-sea environment. This conclusion is further supported by the comparison of the specific gene families (referring to the unique gene families, which present in only one genome) in different *Shewanella* genomes (Fig. 2B and Fig. S5). Compared with other deep-sea *Shewanella* strains, YLB-08 and YLB-09 exhibited almost no specific gene families (Fig. 2B and Fig. S5). Besides, the comparative analysis indicated that there is no gene family that commonly exists in bathypelagic *Shewanella*, further support the notion that diverse adaptation strategies were adopted by bathyal microbes. Interestingly, five gene families, which were related to COG-M (cell wall/membrane/envelope biogenesis) and COG-E (amino acid transport and metabolism) were identified exclusively in abyssal/hadal strains (Table S3). Specifically, a gene

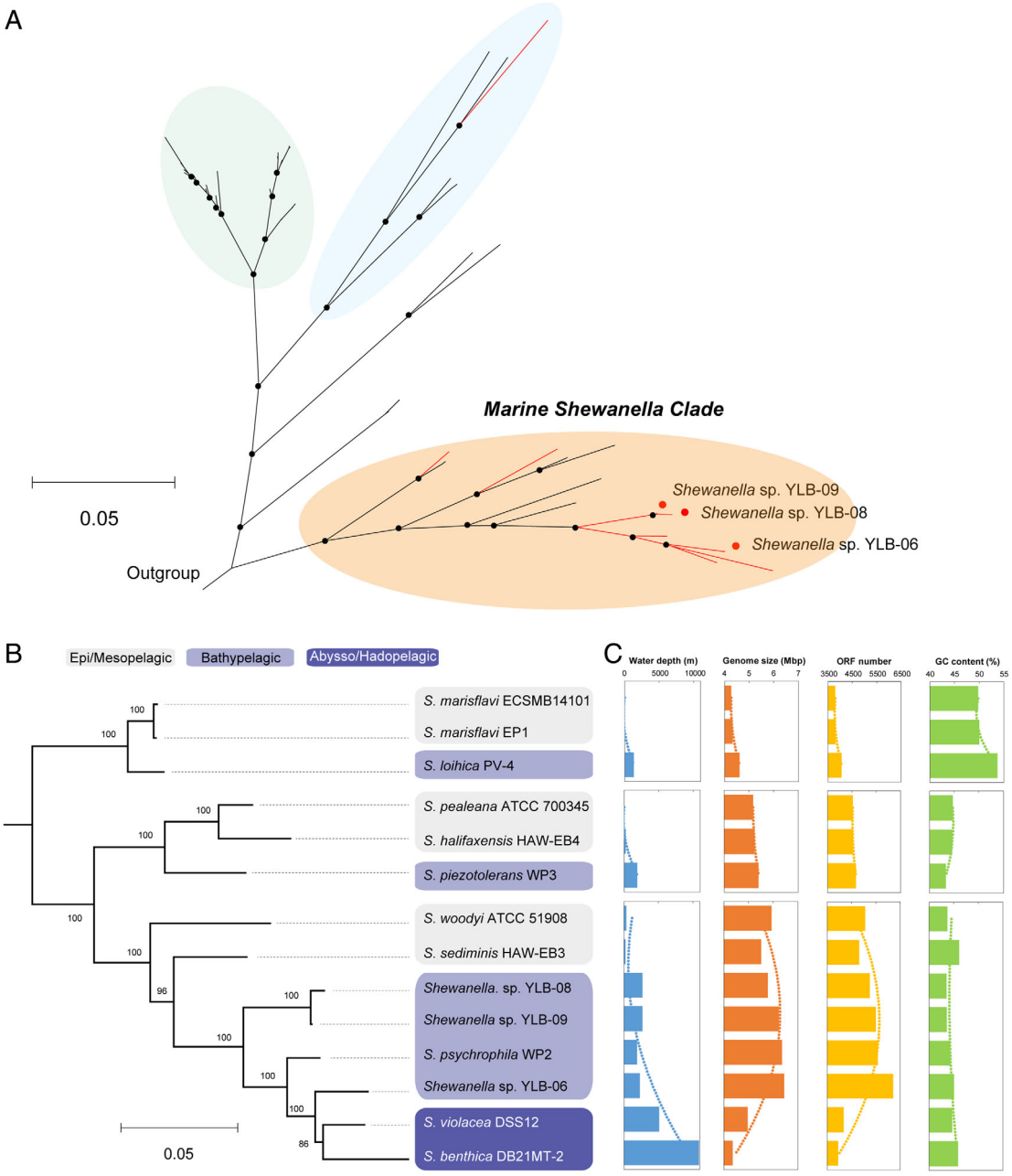

Fig 1. (A) Phylogenetic tree of *Shewanella* genomes constructed using a concatenated alignment of 73 conserved marker genes. Prominent clades are shaded in different colours, and the branches of deep-sea species are labelled in red. Nodes with support values higher than 0.95 are indicated with black circles, and the three strains that were isolated and characterized in this study are indicated with red dots. The complete phylogenetic tree is provided in Fig. S2. (B, C) Overview of the phylogeny and genomic features of the marine *Shewanella* clade. Habitat groups are shaded in different colours according to their isolation sites (grey, epipelagic or mesopelagic zone; light blue, bathypelagic zone; dark blue, abyssopelagic or hadal zone).

family involved in polyamine biosynthesis (GO:0006596), were found in *S. benthica* DB21MT-2 and *S. violacea* DSS12. The polyamine biosynthesis genes have been shown to be abundant in the metagenome of deep-sea sediments and highly expressed at high hydrostatic pressure and low temperature (50 MPa/5°C) (Singh *et al*., 2012; Wang and Sun, 2017). Moreover, polyamines such as trimethylamine N-oxide (TMAO) has been demonstrated to contribute to the adaptation of deep-sea bacterium in counteracting the effect of elevated hydrostatic pressure (Zhang *et al*., 2016; Yin *et al*., 2019). Nevertheless, the distribution of these five gene families in other abyssal and hadal microorganisms and their function in adaptation and survival under

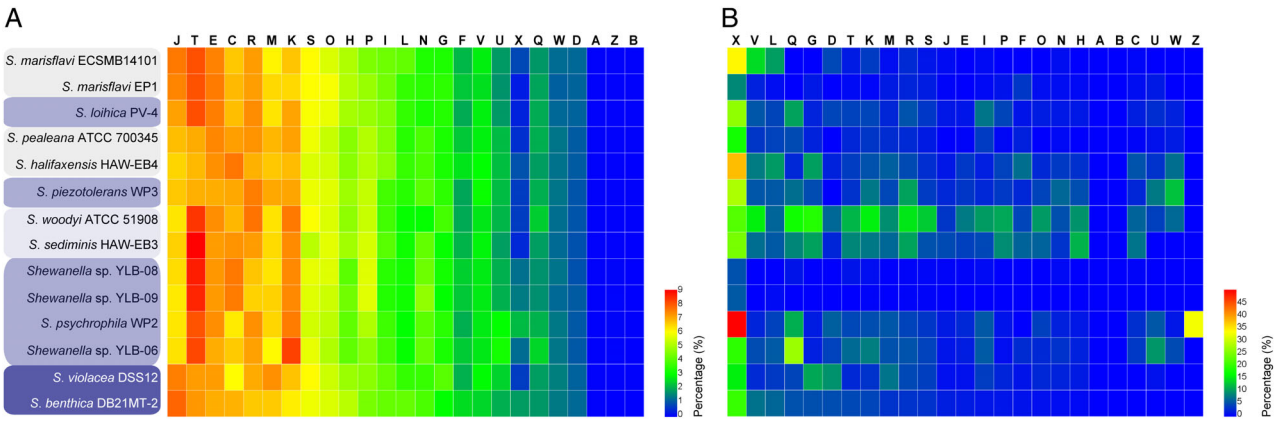

Fig 2. Heat map showing the whole genome (A) and specific gene families (B) composition of 14 marine *Shewanella* strains according to COG (Clusters of Orthologous Groups of proteins) functional categories (http://www.ncbi.nlm.nih.gov/COG/). Abbreviations: J, translation, ribosomal structure and biogenesis; A, RNA processing and modification; K, transcription; L, replication, recombination, and repair; B, chromatin structure and dynamics; D, cell cycle control, cell division, and chromosome partitioning; V, defence mechanisms; T, signal transduction mechanisms; M, cell wall/membrane/envelope biogenesis; N, cell motility; U, intracellular trafficking, secretion and vesicular transport; O, post-translational modification, protein turnover, and chaperones; C, energy production and conversion; G, carbohydrate transport and metabolism; E, amino acid transport and metabolism; F, nucleotide transport and metabolism; H, coenzyme transport and metabolism; I, lipid transport and metabolism; P, inorganic ion transport and metabolism; Q, secondary metabolite biosynthesis, transport, and catabolism; X, mobilome: prophages and transposons; W, extracellular structures; Z, cytoskeleton; R, general function prediction only; S, function unknown.

extreme environmental conditions of the deep ocean await investigation in future studies.

Remarkably, the specific gene families related to COG-X were significantly enriched in most species, indicating that horizontal gene transfer mediated by mobile elements such as bacteriophages plays an important role in generating novel functional genes (Canchaya *et al*., 2003; Touchon *et al*., 2017). In addition, specific gene families related to COG-Q (secondary metabolites biosynthesis, transport and catabolism) were also enriched. For example, *S. piezotolerans* WP3 and *S. loihica* PV-4 exhibit more specific gene families belonging to COG-Q than their shallower companions (Fig. 2B).

Evolutionary history of the Shewanella *strain from the upper ocean to the hadal zone*

The gains and losses of genes reflect the evolutionary processes whereby bacteria adapt to the environment (Abby and Daubin, 2007). The common ancestor of marine *Shewanella* was constructed, and the gained and lost gene families were calculated for each evolutionary node (nodes 1–12) and branch (Fig. 3A; Tables S4 and S5). The gain of gene families dominated the early stages of evolution, and the most recent ancestor (MRA) first experienced a large gene acquisition event, in which 1125 gene families were acquired. From the evolution of the MRA to the first two species clusters, the numbers of gained and lost gene families were relatively similar. However, the loss of gene families dominated in the process of MRA evolution to the third cluster. In the evolution of bathypelagic *Shewanella* strains, gain and loss events occurred simultaneously, resulting in a group of strains with significantly large genomes (Fig. 3A). Interestingly, upon reaching the node of abyssal/hadalpelagic species, a significant loss of gene families was observed (Fig. 3A; Tables S4 and S5), in accordance with the considerable shrinkage of the genome. Furthermore, the loss of gene families in *S. benthica* DB21MT-2 was more significant than that in *S. violacea* DSS12 (902 vs. 428), suggesting that microbes in the hadal biosphere are likely to be subject to higher environmental pressure than those in the abyssal zone. Taken together, the scenario of gene gains and losses indicates that a genome streamlining strategy was adopted by the abyssal and hadal *Shewanella* bacteria to adapt to harsh environmental conditions.

Next, we analysed the key events in the evolutionary process. From node 10 to node 9, corresponding to the key processes in the evolution from shallow to deep oceans, the most abundantly changed gene families were related to COG-T, COG-K and COG-C (Fig. 3B), indicating that these functional genes were important for deep-sea adaptation. For the process between node 7 and node 6, representing the key step in evolution from the bathypelagic ocean to the abyssal/hadal zone, genes related to COG-K and COG-T together with COG-E and COG-N (cell motility) showed significant losses (Fig. 3C). Interestingly, there was no overlap between the gained and lost gene families in these two transitions (Fig. S6), indicating that distinct strategies were adopted by marine *Shewanella* to adapt to new environments. Notably, abyssal/hadal *Shewanella* species are indicated to

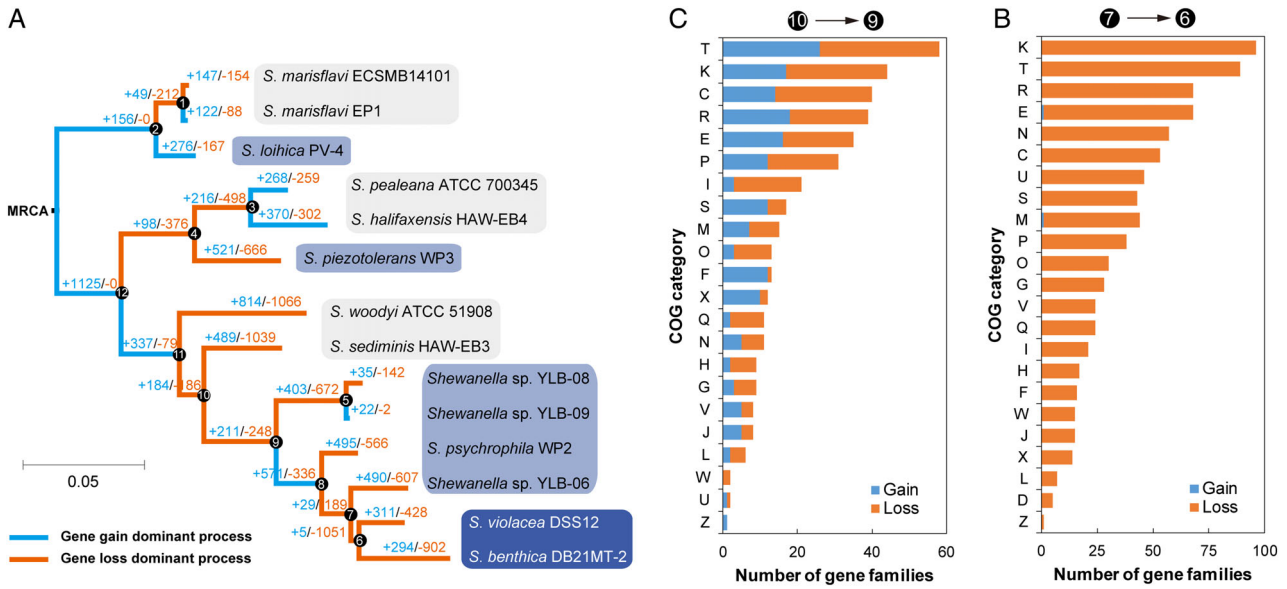

Fig 3. Dynamic evolution of gene families in marine *Shewanella* strains.
(A) The numbers of gained and lost gene families for each node (numbered from 1 to 12) and strain are indicated on the corresponding branch with blue and orange respectively. MRCA: most recent common ancestor.
(B, C) The identified COG functions of the gained and lost genes of the corresponding evolutionary events. The abbreviations of each functional category can be found in the legend of Fig. 2.

exhibit only a single polar flagellum (Fig. 4A), implying an 'energy saving' strategy due to the large amounts of proteins and energy required for flagellar biosynthesis and functioning (Soutourina and Bertin, 2003). Additionally, the numbers of gene families responsible for the utilization of nitrate, dimethyl sulfoxide (DMSO) and TMAO as electron acceptors were significantly reduced in the abyssal/hadal *Shewanella* species (Fig. 4B).

The transcription of the recently gained genes of Shewanella *sp. YLB-06 are repressed at low temperature and high pressure*

To investigate the speciation of *Shewanella* sp. YLB-06, which has the largest known genome among *Shewanella* species, we analysed the functional classification of the gained and lost genes during the process between node 7 and YLB-06. The functional classification showed that

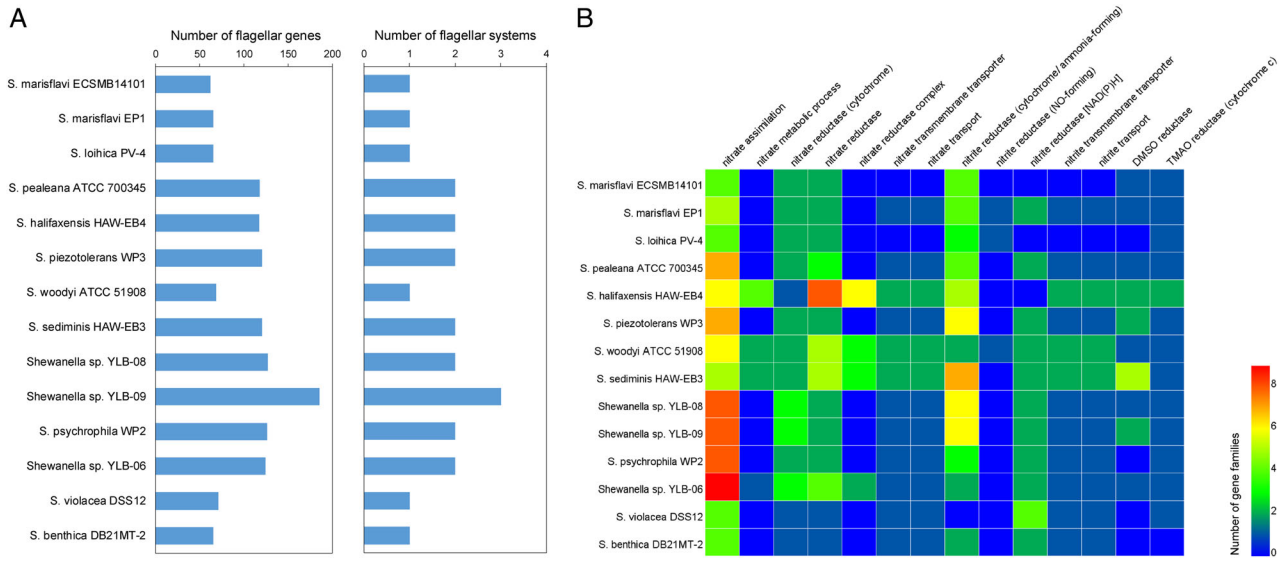

Fig 4. The numbers of flagellar genes/systems (A) and gene families responsible for the utilization of different electron acceptors (B) in 14 marine *Shewanella* strains. The number of flagellar systems was determined based on the gene annotation and manual curation of the completeness of the polar or lateral flagellar system.

the majority of lost genes belonged to COG-T, COG-M and COG-E, while gene families related to COG-K, COG-Q and COG-T were the main categories among the gained genes (Fig. 5A). To explore the extent to which these gained genes participate in environmental adaptation, transcriptomic analysis of *Shewanella* sp. YLB-06 was performed at 23 MPa/4°C and 0.1 MPa/12°C, which correspond to the *in situ* and optimum growth conditions of the strain respectively. To validate the microarray data, seven genes were randomly selected for real-time qPCR analysis. The correlation coefficient (R^2) between the RNA-seq and qPCR data was 0.9599 (Fig. S7), demonstrating that the RNA-seq data were reliable and could be used for follow-up analysis.

We noted that these recently obtained genes tended to occur in clusters, forming hot spots in the genome (Fig. 5B). Subsequently, we analysed the expression of these genes under *in situ* growth conditions in YLB-06. Surprisingly, these clustered distributed genes did not show higher expression levels compared with the other genes in the genome. Conversely, the average FPKM values were 99.08 and 336.28, and the median values were 58.17 and 108.30 for the gained genes and the total genes respectively, indicating that the recently acquired genes exhibit significantly lower (adjusted p-value = 0.232×10^{-29}) transcription levels than the other genes (Fig. 5C). The analysis of the transcriptomic data under the optimal growth conditions showed an average FPKM value of 102.32 and a median of 61.16 for the gained genes, and no significant difference was observed (adjusted p-value = 1) (Fig. 5D and E). Under these two conditions, only three gained genes (all of which are annotated as hypothetical proteins) were found among the differentially expressed genes, which were mainly associated with cellular metabolic functions, cellular processes, cell parts, catalytic activity and binding according to the classification of the GO database (Table S6). Collectively, these results indicate that recently obtained genes are strictly repressed and are not the main differentially expressed genes that respond to changes in environmental factors.

Taken together, this work presents the first analysis of the evolutionary trajectory of a marine microbial group from the surface ocean to the hadal zone. To further determine whether the genome transitions revealed in the *Shewanella* genus are present in other marine bacterial genera, we searched several representative marine

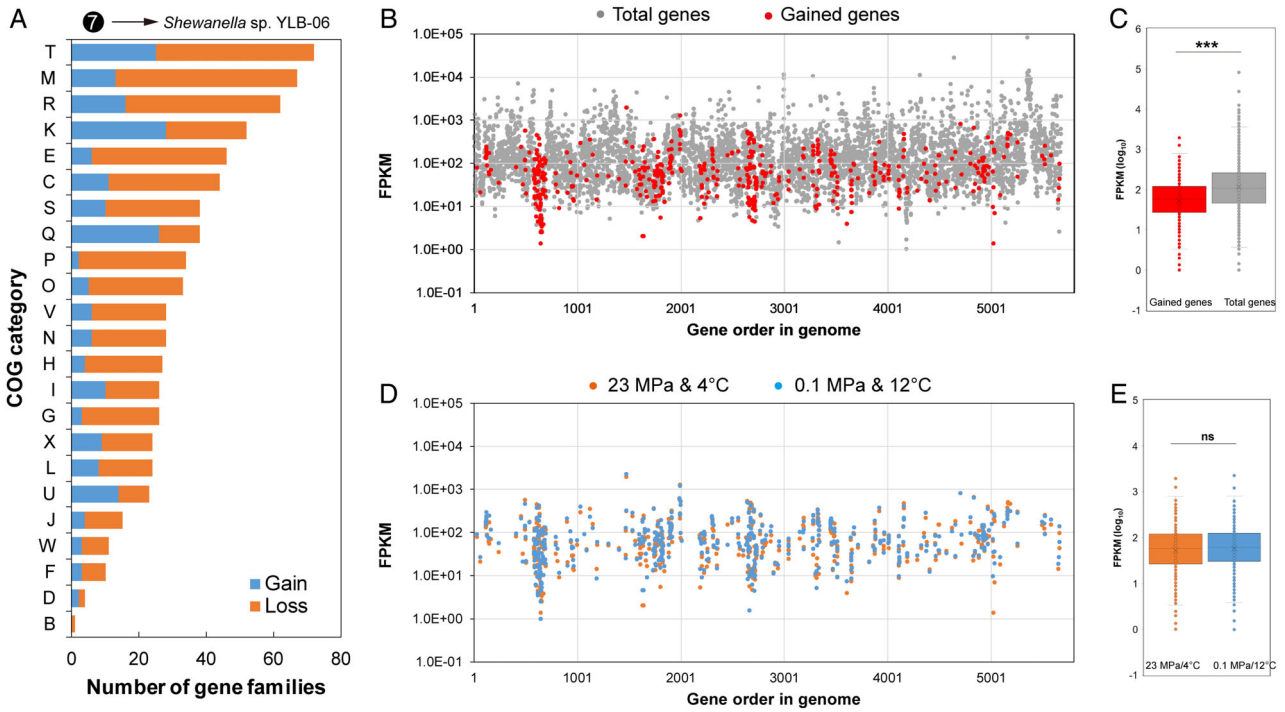

Fig 5. The formation of the largest known genome among *Shewanella* species.
A. The identified COG functions of the gained and lost genes between node 7 and *Shewanella* sp. YLB-06.
B, C. The transcriptional profile and median expression levels measured by the FPKM (fragments per kilobase per million) approach for the total and gained genes of *Shewanella* sp. YLB-06 under *in situ* growth conditions.
D, E. The transcriptional profile and median expression levels measured by the FPKM approach for the gained genes under the *in situ* and optimum growth conditions of *Shewanella* sp. YLB-06. The median expression levels measured by the FPKM approach for the total and gained genes. FPKM values were analysed by the *t* test and Bonferroni correction. ***$p < 0.001$; ns, not significantly different.

bacterial genera, including *Psychromonas*, *Colwellia* and *Moritella*, with available complete genomes and descriptions of water depth, and a similar phenomenon was observed (Fig. 6), suggesting the prevalence of water depth-associated genomic evolution in marine microorganisms. Nevertheless, we acknowledge that it is unclear whether environmental factors other than water depth play a role in this process, due to the limited number of isolated abyssal/hadal microorganisms and the lack of *in situ* physical and chemical parameters. In future studies, we expect that more marine microbial species from different water depths will be isolated and characterized. The acquisition of high-quality whole genome data and accurate environmental parameters and subsequent evolutionary genomics analysis will reveal diverse adaptation strategies and mechanisms, thus providing us with a comprehensive understanding of how the microbial genome responds to environmental changes in marine ecosystems.

Experimental procedures

Isolation, cultivation and DNA extraction of Shewanella *strains*

Three strains, *Shewanella* sp. YLB-06, *Shewanella* sp. YLB-08 and *Shewanella* sp. YLB-09, were isolated from deep-sea sediments in the Southwest Indian Ocean at sites located at E49°43.65′, S37°47.03′ (water depth of 2315 m) and E47°25.27′, S38°45.59′ (water depth of 2699 m). Initially, the samples were enriched in marine broth (MB, BD Difco) at 4°C and 25 MPa for 15 days. These samples were diluted and plated on marine agar (MA, BD Difco). Subsequently, single colonies were picked out and pure cultures were obtained after plate streaking three times successively. All the strains have been deposited at the Marine Culture Collection of China (MCCC) and the Korean Collection for Type Cultures (KCTC), YLB-06 = MCCC 1A12715 = KCTC 62907, YLB-08 = MCCC 1A12718 = KCTC 62909 and YLB-09 = MCCC 1A12717 = KCTC 62910. Genomic DNA was extracted using a Bacterial Genomic Extraction Kit (SBS) following the manufacturer's instructions.

Genome sequencing, assembly and annotation

The genome was sequenced using the Pacific Biosciences (PacBio) RSII single-molecule real-time sequencing platform combined with the Illumina HiSeq system at Shanghai Majorbio Bio-pharm Technology (Shanghai, China). Raw PacBio data were filtered utilizing the Hierarchical Genome Assembly Process version 3.0 (HGAP3) package. Genes were predicted using Glimmer version 3.02 and GeneMarkS (Besemer *et al*., 2001) and annotated through BlastP (BLAST 2.2.28+) searches in the NCBI non-redundant (Nr), String (http://string-db.org/), COG (Galperin *et al*., 2015) and KEGG (Kanehisa *et al*., 2004) databases. rRNAs and tRNAs were predicted using RNAmmer 1.2 (Lagesen *et al*., 2007) and tRNAscan-SE v1.3.1 (Lowe and Eddy, 1997) respectively. Genes of interest were manually evaluated. The DDH estimate value between the two strains was

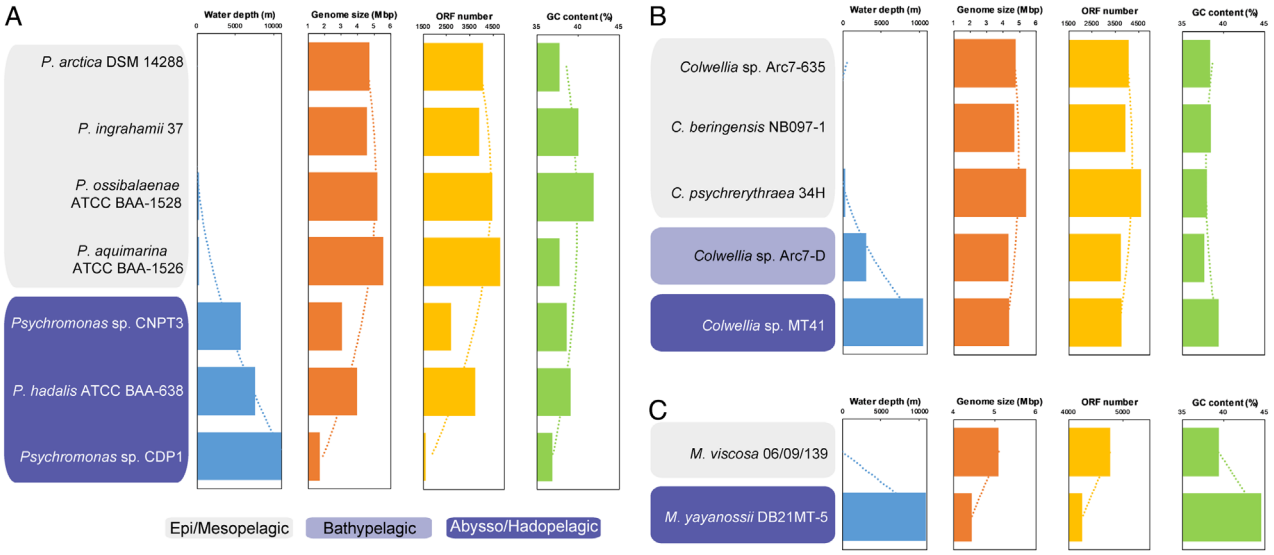

Fig 6. Overview of the genomic features of representative marine bacterial genera, including (A) *Psychromonas*, (B) *Colwellia* and (C) *Moritella*. Habitat groups are shaded in different colours according to their isolation sites (grey, epipelagic or mesopelagic zone; light blue, bathypelagic zone; dark blue, abyssopelagic or hadal zone). Only species with available complete genomes and descriptions of water depth were included in this analysis.

analysed using the genome-to-genome distance calculator (GGDC2.0) with the alignment method of BLAST+ (Auch *et al*., 2010). The ANI between two genomes was calculated by using the web service of EZGenome (http://www.ezbiocloud.net/ezgenome/ani).

Phylogenetic analysis and calculation of genomic characteristics

A total of 41 complete *Shewanella* genomes, including *S*. *benthica* DB21MT-2 from hadal zone, were collected from the NCBI database (retrieved 8 June 2019). A set of 73 conserved marker genes (Lan *et al*., 2014) that were considered not to undergo horizontal gene transfer was used to build the phylogenetic tree of 43 *Shewanella* strains. The homologous sequences were aligned with Clustal Omega 1.2.3 (Sievers *et al*., 2011) and then concatenated. After gaps were deleted manually, phylogenetic trees were generated via maximum likelihood analysis with FastTree 2.1.9 (Price *et al*., 2010) and visualized using MEGA X (Kumar *et al*., 2018). The carbon content of the encoded proteins and the number of nitrogen atoms per residue side chain were calculated using previously described methods (Getz *et al*., 2018).

Gene gain and loss analysis

The protein families of 14 marine *Shewanella* strains were clustered using OrthoMCL 2.0.9 (Li *et al*., 2003) with the following parameters: identity 30%, coverage 50%, E-value 1e-5 and inflation index 1.5. In the present study, the specific gene families refer to the unique gene families, which exist in only one genome among the analysed 14 marine *Shewanella* genomes. The ancestral reconstruction and gene content analysis for the evolutionary tree of 14 marine *Shewanella* strains were calculated using Count (Csürös, 2010) based on Dollo parsimony. The gene functions were annotated in the COG database (Galperin *et al*., 2015), using BLAST with an E-value of 1e−5, identity of 30% and coverage of 50%, and the GO database (Consortium, 2019), using Blast2GO PRO 5.2 (Götz *et al*., 2008).

RNA isolation and real-time qPCR

The *Shewanella* sp. YLB-06 strain was inoculated into 2216E medium, and the culture was collected and frozen in liquid nitrogen immediately when the cells reached the exponential phase. Total RNA extraction, reverse transcription and real-time qPCR were performed as described previously (Jian *et al*., 2016). The primer pairs used to amplify the selected genes via qPCR were designed using Primer Express software (Applied Biosystems, CA, USA). PCR cycling was conducted using 7500 System SDS software (ABI, Foster City, USA) in reaction mixtures with a total volume of 20 μl containing 1× SYBR Green I Universal PCR Master Mix (ABI).

Transcriptomic analysis

Strand-specific transcriptome sequencing was performed at Magigene Biotechnology (Guangdong, China). Briefly, rRNA was removed using the Epicentre Ribo-Zero rRNA Removal Kit (Epicentre, Madison, WI, USA), and the cDNA library was prepared with the NEBNext® Ultra II™ Directional RNA Library Prep Kit for Illumina (NEB, Ipswich, MA, USA) according to the manufacturer's instructions. The initial quantification of the library was carried out by using a Qubit Fluorometer (Life Technologies, Carlsbad, CA, USA), and the insertion fragment size of the library was detected by using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). The effective concentration of the library was quantified accurately by qPCR (effective concentration >2 nM). The different libraries were pooled in the flow cell according to the effective concentration and the requirement of the target offline data volume. After clustering, the Illumina HiSeq sequencing platform (Illumina, San Diego, USA) was used for paired-end sequencing. The raw data were filtered and evaluated with fastp software (Chen *et al*., 2018), and the clean reads were then mapped to the *Shewanella* sp. YLB-06 genome with HISAT software (Kim *et al*., 2015). RSEM (Li and Dewey, 2011) was used to calculate the number of read counts per sample, and the sequencing results were evaluated in terms of quality, alignment, saturation, and the distribution of reads in the reference genome by DGEseq (Wang *et al*., 2010). Gene expression was calculated from the number of reads mapped to the reference gene using the fragments per kilobase per million mapped reads (FPKM) method and analysed by using edgeR (Robinson *et al*., 2010). Differential expressed genes were identified with the following standards: false discovery rate <0.05 and fold change ≥2 of FPKM values between two samples.

Statistical analysis

The two-sample *t*-test was used to determine if two population means were equal. The Bonferroni correction was applied to adjust the type I error to 0.05. The statistical software R 3.6.1 (https://www.r-project.org/) was used for computation.

Availability of data

The sequence of 16S rRNA of *Shewanella* sp. YLB-06, *Shewanella* sp. YLB-08 and *Shewanella* sp. YLB-09 are available in the GenBank, under accession number

MG913996, MG913998 and MG913999 respectively. The complete genome sequences of these three *Shewanella* strains have been deposited at GenBank under the accession number CP041614, CP045503 and CP045427 respectively. The transcriptomic data from the current study have been deposited in the NCBI SRA under the project ID PRJNA579266.

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References

Abby, S., and Daubin, V. (2007) Comparative genomics and the evolution of prokaryotes. *Trends Microbiol* **15**: 135–141.

Aono, E., Baba, T., Ara, T., Nishi, T., Nakamichi, T., Inamoto, E., Toyonaga H., Hasegawa M., Takai Y., Okumura Y., Baba M., Tomita M., Kato C., Oshima T., Nakasone K., Mori H. (2010) Complete genome sequence and comparative analysis of *Shewanella violacea*, a psychrophilic and piezophilic bacterium from deep sea floor sediments. *Mol Biosyst* **6**: 1216–1226.

Auch, A.F., Klenk, H.-P., and Göker, M. (2010) Standard operating procedure for calculating genome-to-genome distances based on high-scoring segment pairs. *Stand Genomic Sci* **2**: 142–148.

Besemer, J., Lomsadze, A., and Borodovsky, M. (2001) GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. *Nucleic Acids Res* **29**: 2607–2618.

Blankenship-Williams, L.E., and Levin, L.A. (2009) Living deep: a synopsis of Hadal trench ecology. *Mar Technol Soc J* **43**: 137–143.

Boeuf, D., Edwards, B.R., Eppley, J.M., Hu, S.K., Poff, K.E., Romano, A.E., Caron D.A., Karl D.M., DeLong E. (2019) Biological composition and microbial dynamics of sinking particulate organic matter at abyssal ocean. *Proc Natl Acad Sci U S A* **116**: 11824–11832.

Canchaya, C., Fournous, G., Chibani-Chennoufi, S., Dillmann, M.-L., and Brüssow, H. (2003) Phage as agents of lateral gene transfer. *Curr Opin Microbiol* **6**: 417–424.

Chen, S., Zhou, Y., Chen, Y., and Gu, J. (2018) Fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **34**: i884-i890.

Consortium, T.G.O. (2019) The gene ontology resource 20 years and stillGOing strong. *Nucleic Acids Res* **47**: D330–D338.

Csürös, M. (2010) Count: evolutionary analysis of phylogenetic profiles with parsimony and likelihood. *Bioinformatics* **26**: 1910–1912.

Eloe, E.A., Malfatti, F., Gutierrez, J., Hardy, K., Schmidt, W. E., Pogliano, K., Pogliano J., Azam F., Bartlett D.H. (2011) Isolation and characterization of a psychropiezophilic alphaproteobacterium. *Appl Environ Microbiol* **77**: 8145–8153.

Fang, Y., Wang, Y., Liu, Z., Dai, H., Cai, H., Li, Z., *et al*. (2019) Multilocus sequence analysis, a rapid and accurate tool for taxonomic classification, evolutionary relationship determination, and population biology studies of the genus *Shewanella*. *Appl Environ Microbiol* **85**: e02153-02118.

Fredrickson, J.K., Romine, M.F., Beliaev, A.S., Auchtung, J. M., Driscoll, M.E., Gardner, T.S., Nealson K.H., Osterman A.L., Pinchuk G., Reed J.L., Rodionov D.A., Rodrigues J.L.M., Saffarini D.A., Serres M.H., Spormann A.M., Zhulin I.B., Tiedje J.M. (2008) Towards environmental systems biology of *Shewanella*. *Nat Rev Microbiol* **6**: 592–603.

Galperin, M.Y., Makarova, K.S., Wolf, Y.I., and Koonin, E.V. (2015) Expanded microbial genome coverage and improved protein family annotation in the COG database. *Nucleic Acids Res* **43**: D261–D269.

Gao, H., Obraztova, A., Stewart, N., Popa, R., Fredrickson, J.K., Tiedje, J.M., Nealson K.H., Zhou J. (2006) *Shewanella loihica* sp. nov., isolated from iron-rich microbial mats in the Pacific Ocean. *Int J Syst Evol Microbiol* **56**: 1911–1916.

Getz, E.W., Tithi, S.S., Zhang, L., and Aylward, F.O. (2018) Parallel evolution of genome streamlining and cellular bioenergetics across the marine radiation of a bacterial phylum. *MBio* **9**: e01089-01018.

Giovannoni, S.J., Thrash, J.C., and Temperton, B. (2014) Implications of streamlining theory for microbial ecology. *ISME J* **8**: 1553–1565.

Giovannoni, S.J., Tripp, H.J., Givan, S., Podar, M., Vergin, K.L., Baptista, D., Bibbs L., Eads J., Richardson T. H., Noordewier M., Rappé M.S., Short J.M., Carrington J. C., Mathur E.J. (2005) Genome streamlining in a cosmopolitan oceanic bacterium. *Science* **309**: 1242–1245.

Götz, S., García-Gómez, J.M., Terol, J., Williams, T.D., Nagaraj, S.H., Nueda, M.J. et al. (2008) High-throughput functional annotation and data mining with the Blast2GO suite. *Nucleic Acids Res* **36**: 3420–3435.

Hau, H.H., and Gralnick, J.A. (2007) Ecology and biotechnology of the genus *Shewanella*. *Annu Rev Microbiol* **61**: 237–258.

Ibarbalz, F.M., Henry, N., Brandão, M.C., Martini, S., Busseni, G., Byrne, H., Coelho L.P., Endo H., Gasol J.M., Gregory A.C., Mahé F., Rigonato J., Royo-Llonch M., Salazar G., Sanz-Sáez I., Scalco E., Soviadan D., Zayed A.A., Zingone A., Labadie K., Ferland J., Marec C., Kandels S., Picheral M., Dimier C., Poulain J., Pisarev S., Carmichael M., Pesant S., Babin M., Boss E., Iudicone D., Jaillon O., Acinas S.G., Ogata H., Pelletier E., Stemmann L., Sullivan M.B., Sunagawa S., Bopp L., de Vargas C., Karp-Boss L., Wincker P., Lombard F., Bowler C., Zinger L., Acinas S.G., Babin M., Bork P., Boss E., Bowler C., Cochrane G., de Vargas C., Follows M., Gorsky G., Grimsley N., Guidi L., Hingamp P., Iudicone D., Jaillon O., Kandels S., Karp-Boss L., Karsenti E., Not F., Ogata H., Pesant S., Poulton N., Raes J., Sardet C., Speich S., Stemmann L., Sullivan M. B., Sunagawa S., Wincker P. (2019) Global trends in marine plankton diversity across kingdoms of life. *Cell* **179**: 1084–1097.

Ichino, M.C., Clark, M.R., Drazen, J.C., Jamieson, A., Jones, D.O.B., Martin, A.P., *et al*. (2015) The distribution of benthic biomass in hadal trenches: a modelling approach to investigate the effect of vertical and lateral organic matter transport to the seafloor. *Deep-Sea Res I Oceanogr Res Pap* **100**: 21–33.

Jamieson, A. (2015) *The Hadal Zone: Life in the Deepest Oceans*. UK: Cambridge University Press.

Jamieson, A.J. (2001) Ecology of deep oceans: Hadal trenches. In *eLS*, Chichester, UK: John Wiley & Sons.

Jamieson, A.J., Fujii, T., Mayor, D.J., Solan, M., and Priede, I.G. (2010) Hadal trenches: the ecology of the deepest places on Earth. *Trends Ecol Evol* **25**: 190–197.

Jian, H., Xu, G., Gai, Y., Xu, J., and Xiao, X. (2016) The histone-like nucleoid structuring protein (H-NS) is a negative regulator of the lateral flagellar system in the deep-sea bacterium *Shewanella piezotolerans* WP3. *Appl Environ Microbiol* **82**: 2388–2398.

Kanehisa, M., Goto, S., Kawashima, S., Okuno, Y., and Hattori, M. (2004) The KEGG resource for deciphering the genome. *Nucleic Acids Res* **32**: D277-D280.

Kim, D., Langmead, B., and Salzberg, S.L. (2015) HISAT: a fast spliced aligner with low memory requirements. *Nat Methods* **12**: 357–360.

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

Kusube, M., Kyaw, T.S., Tanikawa, K., Chastain, R.A., Hardy, K.M., Cameron, J., and Bartlett, D.H. (2017) *Colwellia marinimaniae* sp. nov., a hyperpiezophilic species isolated from an amphipod within the challenger deep, Mariana trench. *Int J Syst Evol Microbiol* **67**: 824–831.

Lagesen, K., Hallin, P., Rødland, E.A., Stærfeldt, H.-H., Rognes, T., and Ussery, D.W. (2007) RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* **35**: 3100–3108.

Lan, Y., Morrison, J.C., Hershberg, R., and Rosen, G.L. (2014) POGO-DB—a database of pairwise-comparisons of genomes and conserved orthologous genes. *Nucleic Acids Res* **42**: D625–D632.

Lauro, F.M., Chastain, A., Ferriera, S., Johnson, J., Yayanos, A.A., and Bartlett, D.H. (2013) Draft genome sequence of the Deep-Sea bacterium *Shewanella benthica* strain KT99. *Genome Announc* **1**: e00210-00213.

Lawrence, J.G., and Hendrickson, H. (2005) Genome evolution in bacteria: order beneath chaos. *Curr Opin Microbiol* **8**: 572–578.

Li, B., and Dewey, C.N. (2011) RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* **12**: 323.

Li, L. Jr., C.J.S., and Roos, D.S. (2003) OrthoMCL: identification of Ortholog groups for eukaryotic genomes *Genome Res* **13**: 2178–2189.

Liu, J., Zheng, Y., Lin, H., Wang, X., Li, M., Liu, Y., Yu M., Zhao M., Pedentchouk N., Lea-Smith D.J., Todd J.D., Magill C.R., Zhang W.J., Zhou S., Song D., Zhong H., Xin Y., Yu M., Tian J., Zhang X.H. (2019) Proliferation of hydrocarbon-degrading microbes at the bottom of the Mariana Trench. *Microbiome* **7**: 47.

Lowe, T.M., and Eddy, S.R. (1997) tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res* **25**: 955–964.

Maruyama, A., Honda, D., Yamamoto, H., Kitamura, K., and Higashihara, T. (2000) Phylogenetic analysis of psychrophilic bacteria isolated from the Japan Trench, including a description of the deep-sea species *Psychrobacter pacificensis* sp. nov. *Int J Syst Evol Microbiol* **50**: 835–846.

Nogi, Y., Hosoya, S., Kato, C., and Horikoshi, K. (2004) *Colwellia piezophila* sp. nov., a novel piezophilic species from deep-sea sediments of the Japan Trench. *Int J Syst Evol Microbiol* **54**: 1627–1631.

Nogi, Y., and Kato, C. (1999) Taxonomic studies of extremely barophilic bacteria isolated from the Mariana Trench and description of *Moritella yayanosii* sp. nov., a new barophilic bacterial isolate. *Extremophiles* **3**: 71–77.

Nogi, Y., Kato, C., and Horikoshi, K. (1998) Taxonomic studies of deep-sea barophilic Shewanella strains and description of *Shewanella violacea* sp. nov. *Arch Microbiol* **170**: 331–338.

Nunoura, T., Takaki, Y., Hirai, M., Shimamura, S., Makabe, A., Koide, O., Kikuchi T., Miyazaki J., Koba K., Yoshida N., Sunamura M., Takai K. (2015) Hadal biosphere: insight into the microbial ecosystem in the deepest ocean on earth. *Proc Natl Acad Sci U S A* **112**: E1230–E1236.

Orcutt, B.N., Sylvan, J.B., Knab, N.J., and Edwards, K.J. (2011) Microbial ecology of the dark ocean above, at, and below the seafloor. *Microbiol Mol Biol Rev* **75**: 361–422.

Price, M.N., Dehal, P.S., and Arkin, A.P. (2010) FastTree 2 – approximately maximum-likelihood Treesfor large alignments. *PLoS One* **5**: e9490.

Qin, Q.-L., Li, Y., Sun, L.-L., Wang, Z.-B., Wang, S., Chen, X.-L., *et al*. (2019) Trophic specialization results in genomic reduction in free-living marine *Idiomarina* bacteria. *MBio* **10**: e02545-02518.

Richter, M., and Rosselló-Móra, R. (2009) Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci U S A* **106**: 19126–19131.

Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**: 139–140.

Salcher, M.M., Schaefle, D., Kaspar, M., Neuenschwander, S. M., and Ghai, R. (2019) Evolution in action: habitat transition from sediment to the pelagial leads to genome streamlining in Methylophilaceae. *ISME J* **13**: 2764–2777.

Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W., Lopez R., McWilliam H., Remmert M., Söding J., Thompson J.D., Higgins D.G. (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* **7**: 539.

Singh, P., Raghukumar, C., Verma, A.K., and Meena, R.M. (2012) Differentially expressed genes under simulated deep-sea conditions in the psychrotolerant yeast *Cryptococcus* sp. NIOCC#PY13. *Extremophiles* **16**: 777–785.

Soutourina, O.A., and Bertin, P.N. (2003) Regulation cascade of flagellar expression in Gram-negative bacteria. *FEMS Microbiol Rev* **27**: 505–523.

Stackebrandt, E., Frederiksen, W., Garrity, G.M., Grimont, P. A.D., Kümpfer, P., Maiden, M.C.J., *et al*. (2002) Report of

the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* **52**: 1043–1047.

Sunagawa, S., Coelho, L.P., Chaffron, S., Kultima, J.R., Labadie, K., Salazar, G., Djahanschiri B., Zeller G., Mende D.R., Alberti A., Cornejo-Castillo F.M., Costea P.I., Cruaud C., d'Ovidio F., Engelen S., Ferrera I., Gasol J.M., Guidi L., Hildebrand F., Kokoszka F., Lepoivre C., Lima-Mendez G., Poulain J., Poulos B.T., Royo-Llonch M., Sarmento H., Vieira-Silva S., Dimier C., Picheral M., Searson S., Kandels-Lewis S., Tara Oceans coordinators, Bowler C., de Vargas C., Gorsky G., Grimsley N., Hingamp P., Iudicone D., Jaillon O., Not F., Ogata H., Pesant S., Speich S., Stemmann L., Sullivan M.B., Weissenbach J., Wincker P., Karsenti E., Raes J., Acinas S.G., Bork P., Boss E., Bowler C., Follows M., Karp-Boss L., Krzic U., Reynaud E.G., Sardet C., Sieracki M., Velayoudon D. (2015) Structure and function of the global ocean microbiome. *Science* **348**: 1261359.

Thorell, K., Meier-Kolthoff, J.P., Sjöling, Å., and Martín-Rodríguez, A.J. (2019) Whole-genome sequencing redefines *Shewanella* taxonomy. *Front Microbiol* **10**: 01861.

Toffin, L., Bidault, A., Pignet, P., Tindall, B.J., Slobodkin, A., Kato, C., and Prieur, D. (2004) *Shewanella profunda* sp. nov., isolated from deep marine sediment of the Nankai Trough. *Int J Syst Evol Microbiol* **54**: 1943–1949.

Touchon, M., Sousa, J.A.M.d., and Rocha, E.P. (2017) Embracing the enemy: the diversification of microbial gene repertoires by phage-mediated horizontal gene transfer. *Curr Opin Microbiol* **38**: 66–73.

Wang, F., Wang, P., Chen, M., and Xiao, X. (2004) Isolation of extremophiles with the detection and retrieval of *Shewanella* strains in deep-sea sediments from the west Pacific. *Extremophiles* **8**: 165–168.

Wang, H., and Sun, L. (2017) Comparative metagenomics reveals insights into the deep-sea adaptation mechanism of the microorganisms in Iheya hydrothermal fields. *World J Microbiol Biotechnol* **33**: 86.

Wang, L., Feng, Z., Wang, X., Wang, X., and Zhang, X. (2010) DEGseq: an R package for identifying differentially expressed genes from RNA-seq data. *Bioinformatics* **26**: 136–138.

Wang, Y., Huang, J.-M., Cui, G.-J., Nunoura, T., Takaki, Y., Li, W.-L., Li J., Gao Z.M., Takai K., Zhang A.Q., Stepanauskas R. (2019) Genomics insights into ecotype formation of ammonia-oxidizing archaea in the deep ocean. *Environ Microbiol* **21**: 716–729.

Wei, Y., Fang, J., Xu, Y., Zhao, W., and Cao, J. (2018) *Corynebacterium hadale* sp. nov. isolated from hadopelagic water of the New Britain Trench. *Int J Syst Evol Microbiol* **68**: 1474–1478.

Xiao, X., Wang, P., Zeng, X., Bartlett, D.H., and Wang, F. (2007) *Shewanella psychrophila* sp. nov. and *Shewanella piezotolerans* sp. nov., isolated from west Pacific deep-sea sediment. *Int J Syst Evol Microbiol* **57**: 60–65.

Yin, Q., Zhang, W., Li, X., Zhou, L., Qi, X., Zhang, C., and Wu, L.-F. (2019) Contribution of trimethylamine N-oxide on the growth and pressure tolerance of deep-sea bacteria. *J Oceanol Limnol* **37**: 210–222.

Yu, L., Tang, X., Wei, S., Qiu, Y., Xu, X., Xu, G., Wang Q., Yang Q. (2019b) Two novel species of the family Bacillaceae: *Oceanobacillus piezotolerans* sp. nov. and *Bacillus piezotolerans* sp. nov., from deep-sea sediment samples of Yap Trench. *Int J Syst Evol Microbiol* **69**: 3022–3030.

Yu, L., Zhou, Z., Wei, S., Xu, X., Wang, Q., Xu, G., Tang X., Yang Q. (2019a) *Marinomonas piezotolerans* sp. nov., isolated from deep-sea sediment of the Yap Trench, Pacific Ocean. *Int J Syst Evol Microbiol* **69**: 739–744.

Zhang, S.-D., Santini, C.-L., Zhang, W., Barbe, V., Mangenot, S., Guyomar, C. et al. (2016) Genomic and physiological analysis reveals versatile metabolic capacity of deep-sea *Photobacterium phosphoreum* ANT-2200. *Extremophiles* **20**: 301–310.

Zhang, W., Cui, X., Chen, L., Yang, J., Li, X., Zhang, C., *et al*. (2019) Complete genome sequence of *Shewanella benthica* DB21MT-2, an obligate piezophilic bacterium isolated from the deepest Mariana Trench sediment. *Mar Genomics* **44**: 52–56.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Circular view of the genomes of the three deep-sea *Shewanella* species that were isolated and sequenced in this study. Circles from interior to exterior represent the GC skew, GC content and coding sequences on the forward and reverse strands, respectively.

Fig. S2. The maximum likelihood tree of 43 *Shewanella* species with complete genome sequences. The tree was created using FastTree 2.1.9 and MEGA X based on the concatenated amino acid sequences of 73 conserved proteins. The tree was rooted with *Escherichia coli* K-12, and multiple species belonging to γ-proteobacteria were used as outgroups. Bootstrap support estimated from 1000 replicates is given below or above each branch.

Fig. S3. The genomic features of the 14 marine *Shewanella* strains. C-ARSC, carbon content in the encoded proteins; NARSC, nitrogen atoms per residue side chain.

Fig. S4. Heat map showing the whole-genome composition of 14 marine *Shewanella* strains according to Gene Ontology (GO) functional categories (http://geneontology.org/). Abbreviations: BP, Biological process; MF: Molecular function; CC: Cellular component.

Fig. S5. Heat map showing the composition of specific genes in 14 marine *Shewanella* strains according to Gene Ontology (GO) functional categories (http://geneontology.org/). Abbreviations: BP, Biological process; MF: Molecular function; CC: Cellular component.

Fig. S6. Venn diagram displaying the relationship between the gained and lost gene families during the evolutionary transition of marine *Shewanella* strains. Node 10 to node 9 and node 7 to node 6 represent the transitions from the epi/mesopelagic to the bathypelagic zone, and from the bathypelagic to the abysso/hadalpelagic zone, respectively.

Fig. S7. Correlation analysis of RNA-seq and RT-qPCR assays. Seven genes showing differences in their expression levels were randomly selected for this assay. The RT-

qPCR $\log_2$ values were plotted against the RNA-seq $\log_2$ values.

Table S1. Characteristics of the 14 analysed marine *Shewanella* genomes.

Table S2. ANI and DDH values between strains YLB-06 and YLB-08 and related *Shewanella* species.

Table S3. The abyssal/hadal *Shewanella* specific gene families.

Table S4. Complete list of gained and lost gene families based on COG annotation.

Table S5. Complete list of gained and lost gene families based on GO annotation.

Table S6. Differentially expressed genes in *Shewanella* sp. YLB-06 at 23 MPa and 4°C compared with 0.1 MPa and 12°C.