

***Shewanella* sp. T2.3D-1.1 a novel microorganism sustaining the Iron cycle in the deep subsurface of the Iberian Pyrite Belt**

Guillermo Mateos ^{1,*}, Adrián Martínez Bonilla ¹, Sofía de Francisco de Polanco ², José M. Martínez ¹, Cristina Escudero ³, Nuria Rodríguez ^{1,3}, Irene Sánchez-Andrea ⁴ and Ricardo Amils ^{1,3}

¹ Centro de Biología Molecular Severo Ochoa, Nicolás Cabrera 1, 28049 Madrid, Spain

² Centro de Investigaciones Biológicas, Ramiro de Maeztu 9, 28040 Madrid, Spain

³ Centro de Astrobiología (CAB-INTA), 28850 Torrejón de Ardoz, Spain

⁴ Laboratory of Microbiology, Wageningen University & Research, Stippeneng, 46708 Wageningen, The Netherlands

* Correspondence: gmateos@cbm.csic.es

Supplementary Figure S1. UBCG tree built with the full set of genomes of the article. GSI index is displayed below branches in black. Distance is displayed on top of each branch in green. *Shewanella* T2.3D-1.1 has been highlighted with bold font.

Supplementary Figure S2. MEME graphical results of the motifs present in the BtuB proteins from *Shewanella* T2.3D-1.1 compared to *Shigella dysenteriae* (RIH4623.1) and *Escherichia coli* (CAD6020855.1).

Supplementary Figure S3. Phylogenetic tree of all available BtuB *Shewanella* sequences in the GenBank database with CLUSTAL-OMEGA. It also includes reference proteins from *E. coli* and *S. dysenteriae*. Distances are displayed as number on every branch.

Supplementary Table S1. Genomes obtained from the GenBank database that have been used in the different analyses of this article. All the type species have been marked with a bold superscripted “T”. **Species** column indicates the species of the genome, **Strain** column is for the strain name and the **Assembly Accession** column is for the accession code for the GenBank database.

Supplementary Table S2. Metadata employed for the elaboration of the Pangenome figure for the. This information is displayed in **Figure 2**. The **Name** column contains the strain names for every single *Shewanella putrefaciens* strain employed. The **Isolation** column has the available data on the source of each microorganism, where NA stands for Not Available. The **dddH** column has the data for the Digital DNA-DNA Hybridization comparing *Shewanella* sp. T2.3D-1.1 to every single genome annotated as *Shewanella putrefaciens* available in the GenBank database.

Supplementary Table S3. Raw results of the variant calling analysis using *Shewanella putrefaciens* CN-32 as the reference against *Shewanella* T2.3D-1.1.

Supplementary Table S4. Results from the variant calling analysis classified and mapped to the *Shewanella putrefaciens* CN-32 chromosome. Columns with the “variant_” prefix indicate the type of variant that affects a particular gene or sequence.

Supplementary Table S5. ClusterProfiler classification of genes according to the Kegg.db on R. Pathways that are missing information were obtained through the KEGG Mapper tool using the genome of *Shewanella* sp T2.3D-1.1 and comparing it to *Shewanella putrefaciens* CN-32.

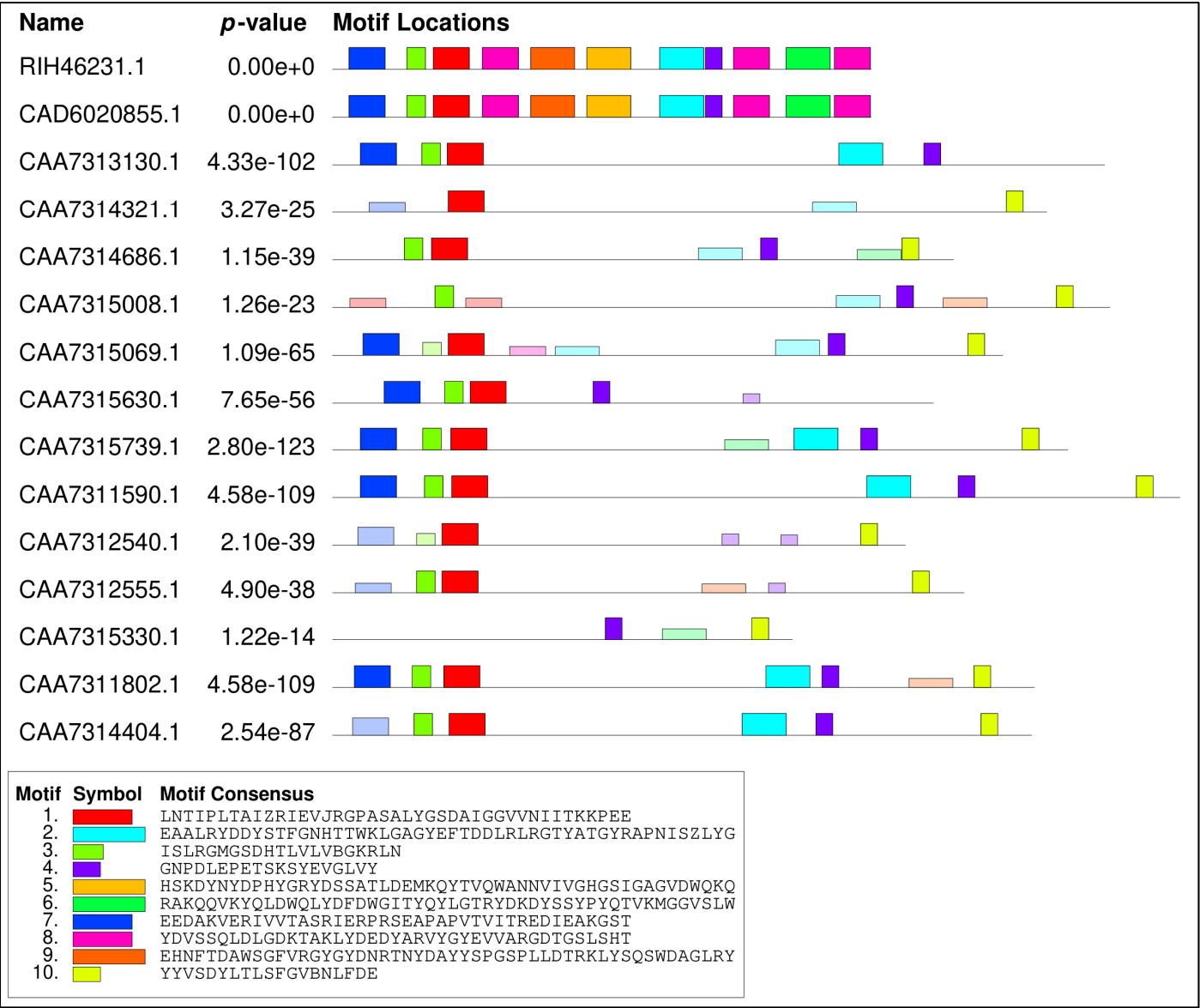
Supplementary Table S6. Genes found in *Shewanella* T2.3D-1.1's genome that have been mentioned throughout the article. Different row colours are determined by the associated pathway from the last column.

Supplementary Table S7. Genes that have 4 or more copies in the chromosome of *Shewanella* T2.3D-1.1.

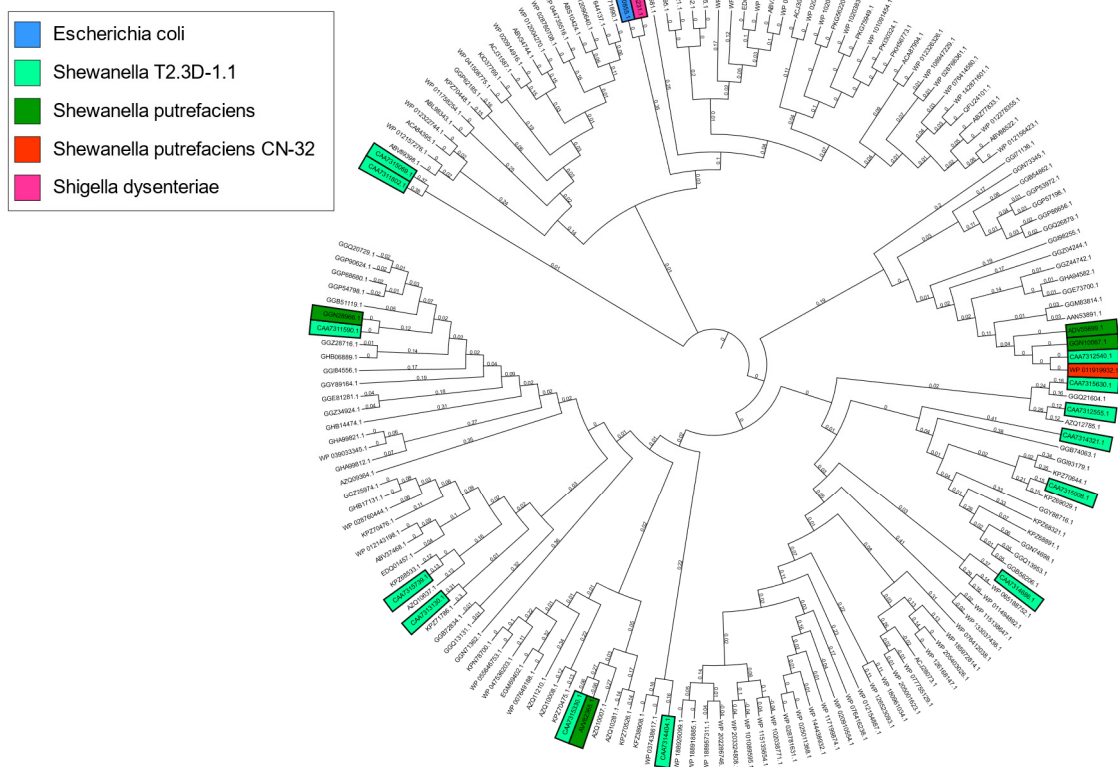
Supplementary Table S8. ANI values calculated with *Shewanella* sp. T2.3D-1.1 as the reference against type strain genomes from the *Shewanella* genus.

Supplementary Table S9. dDDH results according to the three different formulas available on the web server. Each colour indicates different formulas from the online server used in the calculation. *Shewanella* sp. T2.3D-1.1 was set as the reference genome against type strains from the *Shewanella* genus.

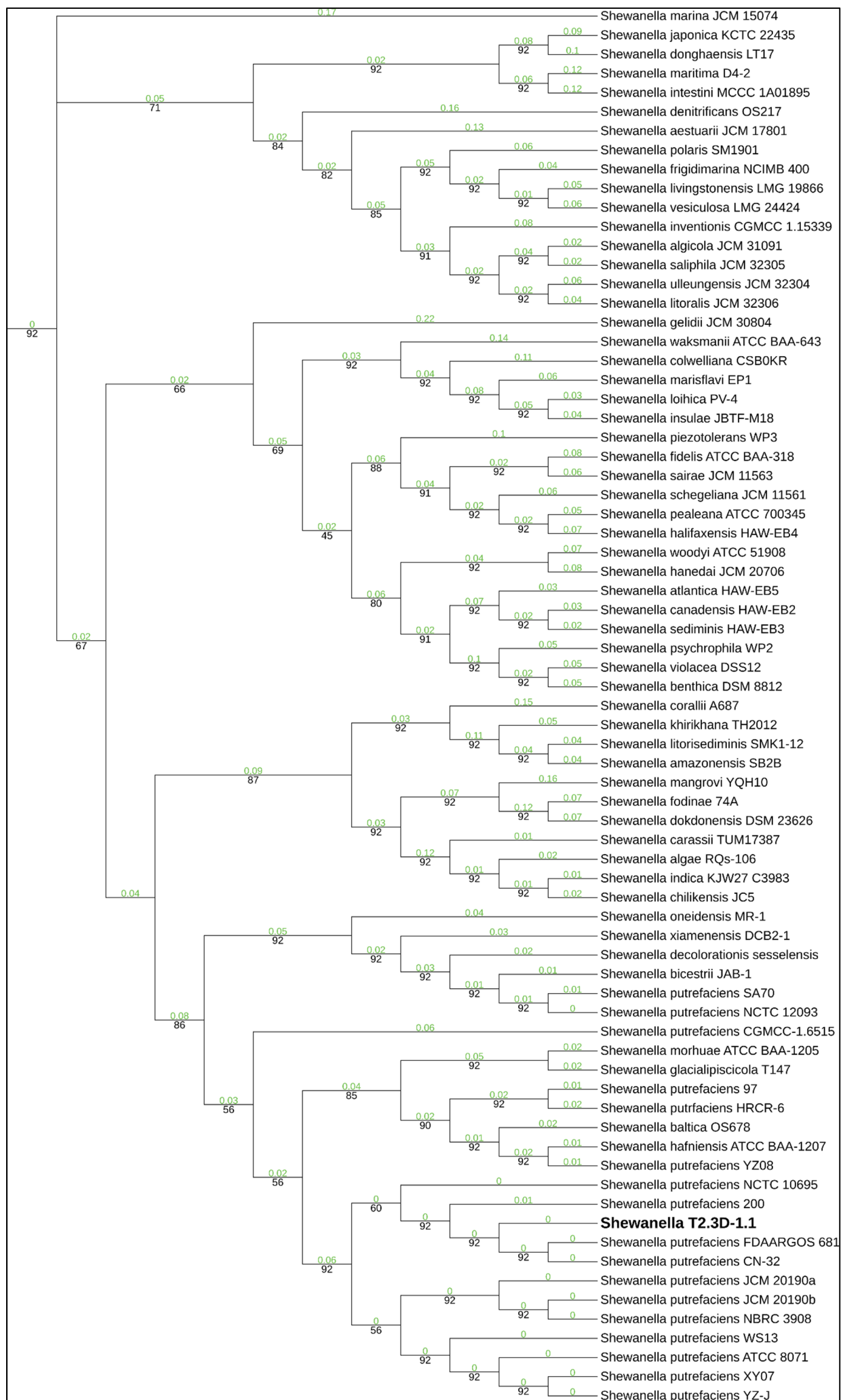
Supplementary Table S10. KEGG mapper information on each for every gene found from *S. putrefaciens* CN-32 with variants. It contains the KEGG metabolic pathway in which each gene is mapped and the identifier and the name of the gene with a hyperlink to the database for further information.



Supplementary Figure S1. MEME graphical results of the motifs present in the BtuB proteins from *Shewanella* T2.3D-1.1 compared to *S.dysenteriae* (RIH4623.1) and *E.coli* (CAD6020855.1).



Supplementary figure S2. Phylogenetic tree of all available BtuB *Shewanella* sequences in the GenBank database with CLUSTAL-OMEGA. It also includes reference proteins from *E. coli* and *S. dysenteriae*. Distances are displayed as number on every branch.



Supplementary Figure S3. UBCG tree built with the full set of genomes of the article. GSI index is displayed below branches in black. Distance is displayed on top of each branch in green. *Shewanella* T2.3D-1.1 has been highlighted with bold font.