

Draft Genome Sequence of Anammox Bacterium “*Candidatus Scalindua brodae*,” Obtained Using Differential Coverage Binning of Sequencing Data from Two Reactor Enrichments

Daan R. Speth,^a Lina Russ,^a Boran Kartal,^a Huub J. M. op den Camp,^a Bas E. Dutilh,^{b,c,d} Mike S. M. Jetten^{a,e}

Ecological Microbiology, IWWR, Radboud University Nijmegen, Nijmegen, The Netherlands^a; Theoretical Biology and Bioinformatics, Utrecht University, Utrecht, The Netherlands^b; Centre for Molecular and Biomolecular Informatics, Radboud University Medical Centre, Nijmegen, The Netherlands^c; Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil^d; Department of Biotechnology, Delft University of Technology, Delft, The Netherlands^e

We present the draft genome of anammox bacterium “*Candidatus Scalindua brodae*,” which at 282 contigs is a major improvement over the highly fragmented genome assembly of related species “*Ca. Scalindua profunda*” (1,580 contigs) which was previously published.

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Address correspondence to Daan R. Speth, d.speth@science.ru.nl.

Anammox bacteria are major players in the global nitrogen cycle, capable of anaerobically oxidizing ammonium to dinitrogen gas, using nitrite as the electron acceptor (1). All currently known anammox bacteria form the monophyletic order *Brocadiales* within the phylum *Planctomycetes* (2). Until now, draft genomes of four anammox species have been reported (3–6). The genome assemblies of “*Candidatus Kuenenia stuttgartiensis*” and “*Ca. Jettenia caeni*” are in 5 and 4 contigs, respectively, whereas the draft genome of “*Ca. Brocadia fulgida*” (411 contigs) is fragmented and the “*Ca. Scalindua profunda*” draft genome (1,580 contigs) is highly fragmented.

Despite advances in culturing techniques, no pure culture of anammox bacteria exists. This restricts genome-sequencing efforts to metagenomic sequencing and binning (7, 8). Here we employed a differential coverage binning approach (9) to increase the confidence of the binning result. We combined sequencing data from Russ et al. (10) with sequencing data from the enrichment culture used as seed for the experimental reactor described in Russ et al. (10). The raw sequence data are available in DDBJ/EMBL/Genbank under accession no. ERX443234 and SRX719339. DNA isolation and sequencing of both enrichments was performed as described previously, using the Powersoil DNA isolation kit (Mo-Bio, Carlsbad, CA, USA) according to the manufacturer’s instructions and the Ion Torrent 200 bp workflow (10).

All data were co-assembled using the CLC genomics workbench (v7.0.4, CLCbio, Aarhus, Denmark) *de novo* assembler, using word size 35 and bubble size 5,000. The obtained contigs were binned with a workflow modified from Albertsen et al. (9) using custom scripts available at http://www.github.com/dspeth/bioinfo_scripts. The binned “*Ca. Scalindua brodae*” genome consisted of 282 contigs and was annotated using Prokka 1.10 (11) followed by manual curation. Frameshifts were corrected using the CLC genomics workbench (v7.0.4, CLCbio, Aarhus, Denmark) and a Perl script available at http://www.github.com/dspeth/bioinfo_scripts. The

completeness (>92%) of the draft genome was assessed using CheckM (12). The contigs have a total length of 4.1 Mb, average G+C content of 39.6%, and encode 4,016 genes, 39 tRNAs, and 1 rRNA operon.

Hydrazine is a key intermediate in anammox metabolism and the enzymes involved in its turnover are unique to anammox bacteria. The fusion of subunits B and C of the hydrazine synthase operon (*hzsBC*), earlier reported for “*Ca. Scalindua profunda*” (6), was confirmed and additionally the fused genes seemed to have undergone duplication. The presence of two copies of *hzsBC* prohibited resolving their genomic location without mate pair information. As a result, the hydrazine synthase BC gene is present on a separate contig. The hydrazine dehydrogenase is also present in two copies, but their genomic location could be resolved based on sequence difference.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. [JRYO000000000](https://www.ncbi.nlm.nih.gov/nuccore/JRYO000000000). The version described in this paper is the first version, [JRYO010000000](https://www.ncbi.nlm.nih.gov/nuccore/JRYO010000000).

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