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Archaeal ammonia oxidisers are abundant in acidic, coarse-textured Australian soils

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Introduction

In the study of acid pasture soils from south west Western Australia the diversity of the *amoA* gene from ammonia oxidising archaea was examined using DGGE (figure s1). This accessory publication describes the results of the sequencing and phylogenetic analysis of the dominant bands from the DGGE gel.

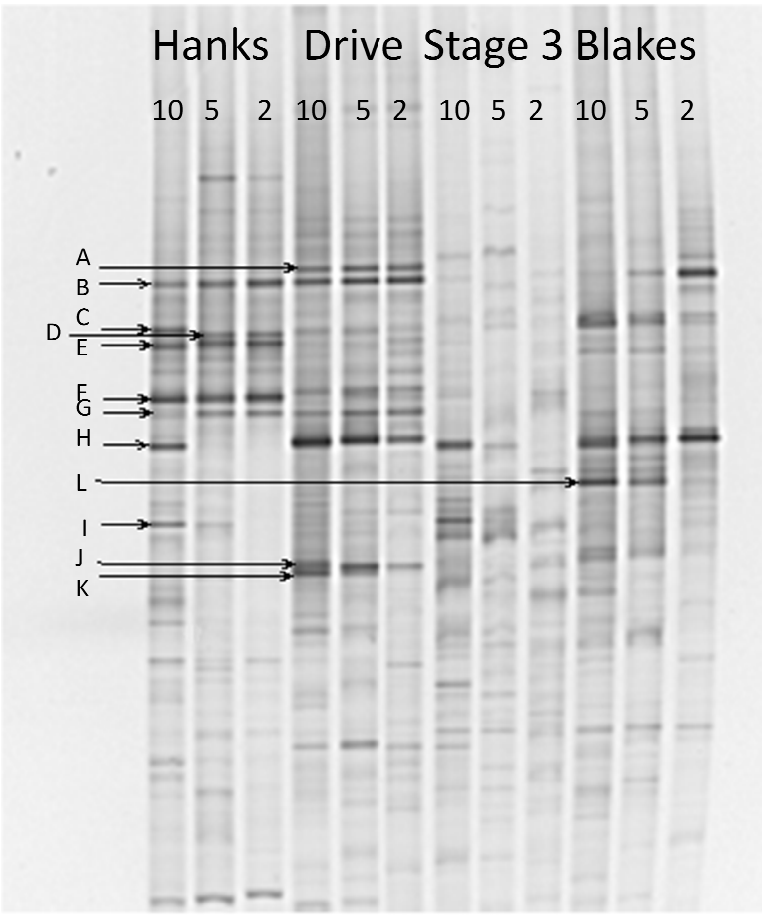

Figure s1: Image of DGGE gel for archaeal amoA genes. Site identification is given at the top of each lane. Numbers indicate depth in centimetres at the bottom of the sample core within each site. Letters identify bands excised for sequencing.

Materials and methods

PCR-DGGE protocols were followed as described in "Archaeal ammonia oxidisers are abundant in acidic, coarse-textured Australian soils".
A selection of the dominant bands (figure s1) was made based on their intensity. Individual bands were extracted from the gel with a scalpel and eluted in 50ul of sterile milliQ water overnight at 4^{o}C. One in ten dilutions were used for subsequent re-amplification and DGGE. This was repeated until single bands were generated for each of the 11 bands selected.

DNA fragments from the bands were cleaned with Promega Wizard® SV Gel and PCR Clean Up system (Promega, Alexandria, NSW, Australia) as per manufacturer's instructions and cloned into TOPO TA cloning vector (Invitrogen, Mulgrave, Vic, Australia) with minor modifications to the protocol. Five colonies per band were selected to verify each band was representative of an individual molecular operationaltaxonomic unit and sent to the Australian Genome Research Facility (Brisbane, Australia) for plasmid purification and PCR sequencing.

Sequences were aligned and edited in BioEdit (version 7.0.0, Ibis Biosciences, Carlsbad, California, USA). The similarity of the sequences to existing sequences was determined using the Basic Local Alignment Search Tool (BLAST) (Altschul, Gish *et al.* 1990). The closest match for each of the DGGE band sequences were downloaded from GenBank and added to the analysis; the amoA sequences for several isolated AOA were also added for reference (Benson, Karsch-Mizrachi *et al.* 2011). All sequences were aligned in MEGA5 (Tamura, Peterson *et al.* 2011). A phylogenetic tree was created using the neighbour joining method with bootstrap values calculated from 1000 re-sampled datasets.

Results and Discussion

The BLAST analysis revealed that all of the DGGE band clones were closely related to uncultured Crenarchaeota. This is not surprising give the very small number of AOA that have been isolated. The phylogenetic tree that was produced using the neighbour-joining method is shown in Figure s2. Nine of the clones from the DGGE analysis clustered within the soil cluster of the AOA while the remaining three grouped with the marine cluster.

The clones from this analysis were more closely related to uncultured AOA from northern hemisphere sites than those from Australian sites that have been previously deposited in the Genbank database. This is likely to be a result from the low level of sampling intensity conducted from Australian sites compared with efforts internationally.

The sequences retrieved from the DGGE bands in this study have been submitted to Genbank with accession numbers JQ406891-JQ406902 (Benson, Karsch-Mizrachi *et al.* 2011).

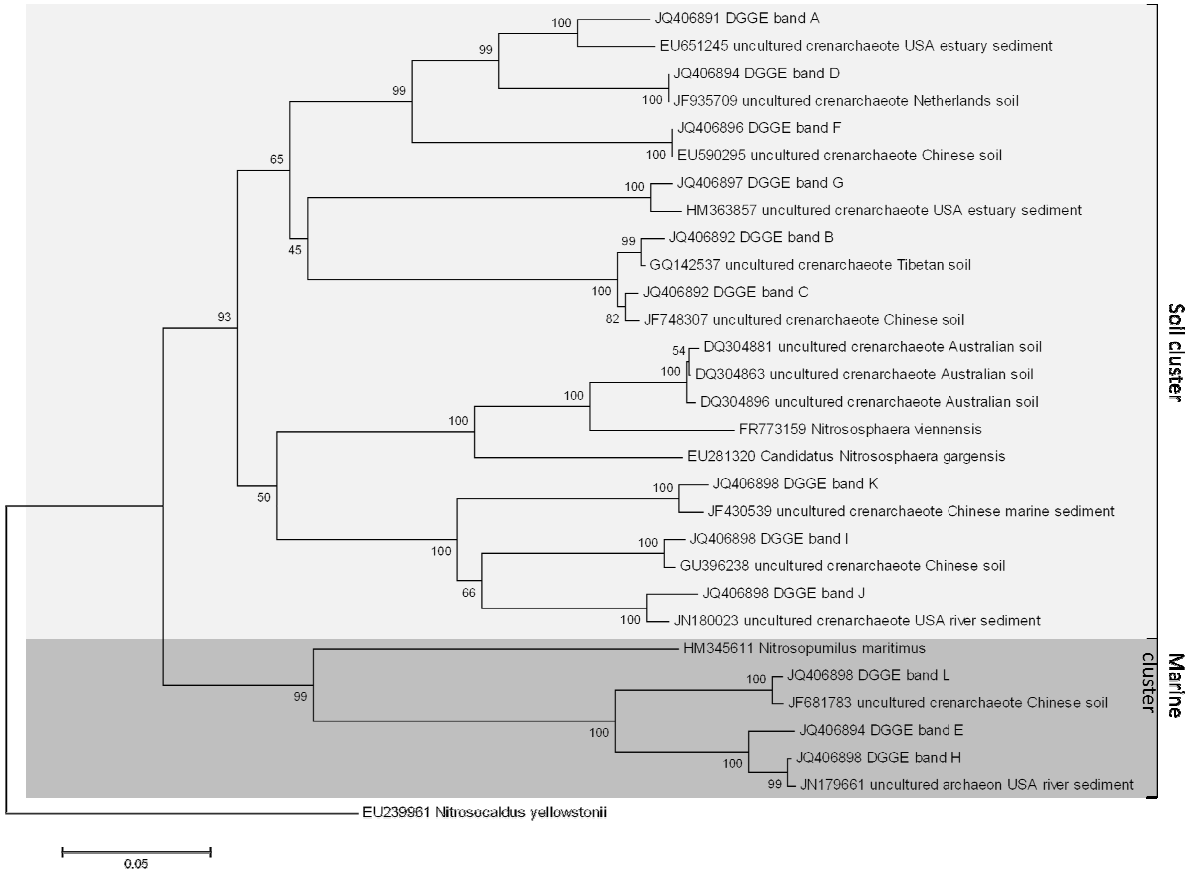

Figure s2: Phylogenetic relationships between dominant AOA from DGGE analysis, a variety of amoA from uncultured AOA and isolated AOA. All sequences were retrieved from GenBank except those that were analysed in this study. The tree is based on > 600 base pairs and was constructed using the neighbour joining method in MEGA5. Numbers at nodes are bootstrap values from 1000 re-sampled data points. The scale bar represents 0.05 nucleotide changes per position.

References

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Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* **28**(10), 2731-2739.