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Nitrosotalea devaniterrae gen. nov., sp. nov. and Nitrosotalea sinensis sp. nov., two acidophilic ammonia oxidising archaea isolated from acidic soil, and proposal of the new order Nitrosotaleales ord. nov. within the class Nitrososphaeria of the phylum Nitrososphaerota

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1 *Nitrosotalea devaniterrae* gen. nov., sp. nov., and *Nitrosotalea sinensis* sp. nov., two
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3 new order *Nitrosotaleales* ord. nov. within the class *Nitrososphaeria* of the phylum
4 *Nitrososphaerota*

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18 **Keywords:** *Nitrosotalea*, soil, archaeal ammonia oxidiser, nitrification, ammonia oxidation,
19 acidophile

20 **Accession numbers:** NCBI accession numbers for genome sequences are PRJEB10948
21 (complete) for Nd1^T and GCF_900143675 (draft) for Nd2^T. NCBI accession numbers for 16S
22 rRNA gene sequences are JN227488 for Nd1^T and KJ540205 for Nd2^T.

23 **Abbreviations:** AOA, ammonia-oxidising archaea; AOB, ammonia-oxidising bacteria;
24 *amoA*, ammonia monooxygenase sub-unit A; ANI, average nucleotide identity; ATU,
25 allylthiourea; DAPI, 4',6-diamidino-2-phenylindole; DCD, dicyandiamide; DOPE-FISH,
26 double labelling of oligonucleotide probes for fluorescence in situ hybridization; GDGT,
27 glycerol dibiphytanyl glycerol tetraethers; GTDB, Genome Taxonomy Database; GTGT,
28 glycerol trialkyl glycerol tetraethers; IPL, intact polar lipids; MeO-AR, methoxy archaeol;
29 MES, 2-(N-morpholino)ethanesulfonic acid.

30 **Abstract**

31 Two obligately acidophilic, mesophilic and aerobic soil ammonia-oxidising archaea were
32 isolated from a pH 4.5 arable sandy loam (UK) and pH 4.7 acidic sulphate paddy soil (China)
33 and designated strains Nd1^T and Nd2^T, respectively. Strains share >99% 16S rRNA gene
34 sequence identity and their genomes are <2 Mb in length, sharing 79% average nucleotide
35 identity, 81% average amino acid identity and a G+C mol% ~37%. Strains are
36 chemolithotrophs that fix carbon dioxide and gain energy by oxidising ammonia to nitrite with
37 no evidence of mixotrophic growth. Neither strain is capable of using urea as a source of
38 ammonia. Strains are non-motile in culture although Nd1^T does possess genes encoding flagella
39 and therefore may be motile under certain conditions. Cells are small angular rods 0.5 - 1 µm
40 in length and grow in the pH range 4.0 – 6.1 and temperature range 20 – 40°C. Strains Nd1^T
41 and Nd2^T are distinct in genomic and physiological features and are assigned as the type strains
42 for the species *Nitrosotalea devaniterrae* sp. nov. (type strain, Nd1^T=NCIMB 15248^T=DSM
43 110862^T) and *Nitrosotalea sinensis* sp. nov. (type strain, Nd2^T=NCIMB 15249^T=DSM
44 110863^T), respectively, within the genus *Nitrosotalea* gen. nov. The family *Nitrosotaleaceae*
45 fam. nov. and order *Nitrosotaleales* ord. nov. are also proposed officially.

46 **Introduction**

47 Nitrification, the oxidation of ammonia to nitrite and subsequently to nitrate, is a key process
48 within the terrestrial and aquatic nitrogen cycles and in aerobic wastewater treatment processes.
49 The first step in this process, ammonia oxidation to nitrite, was considered to be performed
50 exclusively by ammonia-oxidising bacteria (AOB), following their initial cultivation from soil
51 in 1890 (1). Ammonia oxidation provides energy to these chemolithoautotrophs and their
52 presence and activity have been demonstrated in a wide range of environments. This view of
53 ammonia oxidation was overturned by isolation of the first ammonia-oxidising archaeon,
54 *Nitrosopumilus maritimus* SCM1^T (2), from a tropical marine aquarium, after earlier evidence

55 from marine and soil metagenomes (3,4). All currently isolated ammonia-oxidising archaea
56 (AOA) are autotrophic and belong to the phylum *Nitrososphaerota* (5) (formally
57 *Thaumarchaeota* (6) and within the *Thermoproteota* phylum in GTDB taxonomy (7)), within
58 the *Archaea*. Extensive molecular ecology studies have provided evidence for ubiquity of AOA
59 in terrestrial and aquatic environments. They appear to dominate ammonia-oxidising activity
60 in marine ecosystems (8,9) and contribute to ammonia oxidation in soil systems together with
61 AOB and complete ammonia-oxidising *Nitrospira* (10).

62 Fundamental physiological differences between members of the archaeal and bacterial domains
63 stimulated investigation of potential niche specialisation and differentiation of AOA and AOB
64 (11,12) and physiological diversity within these two groups has led to their co-existence in the
65 majority of soils. All isolated betaproteobacterial AOB grow optimally at near neutral pH
66 although some *Nitrospira* strains can demonstrate growth down to pH 5.0 (13,14) and an
67 acid-tolerant gammaproteobacterial AOB has recently been cultivated from soil (15). However,
68 there is strong evidence for significant contributions of AOA to ammonia oxidation in acid
69 soils (16,17). Here we describe the isolation and taxonomic placement of two obligately
70 acidophilic AOA belonging to the class *Nitrososphaeria* that provide a convincing explanation
71 for this observation.

72 **Habitat and isolation**

73 Strains Nd1^T and Nd2^T were both isolated from acidic soils. Strain Nd1^T was enriched from an
74 acidic agricultural soil (loamy sand) sampled from Scotland's Rural College Craibstone
75 Woodlands Field pH plots (latitude/longitude: 57.18 N/2.21 W), with an 8-year crop rotation
76 cycle (18). Soil pH had been maintained at pH 4.5 since 1961 and other soil characteristics are
77 described in Kemp et al. (19) and Paterson et al. (20). Isolation of a pure culture of strain Nd1^T
78 from this enrichment is described in Lehtovirta-Morley et al. (21). Strain Nd2^T was isolated
79 from an acidic sulphate paddy soil (pH 4.7) sampled in Taishan County, Guangdong Province

80 (latitude/longitude: 22.11 N/112.81 E) in Southern China that had been subjected to urea
81 fertilisation and double rice-cropping since 2003 (21).

82 While autotrophic soil ammonia oxidisers can form colonies on solidified medium after several
83 weeks or months (22,23) they are difficult to separate from faster growing heterotrophs.
84 Ammonia oxidisers are therefore typically enriched by inoculation of liquid mineral salts
85 medium, containing ammonium, with soil and incubated for several weeks, with or without
86 shaking. Enrichment of strain Nd1^T was achieved by inoculation of soil (1% w/v) into 100 ml
87 glass bottles containing 50 ml mineral salts medium (18) with 500 µM ammonium chloride
88 and 50 mg l⁻¹ streptomycin, adjusted to pH 4.5 with HCl and incubation at 28°C in the dark
89 without shaking. Ammonia oxidation was assessed by an increase in nitrite concentrations and
90 Nd1^T enriched by routinely transferring 2% of mid-exponential culture (~50 µM nitrate
91 produced). After repeated subcultures in liquid medium to reduce the richness and abundance
92 of contaminating heterotrophs, a highly enriched culture was obtained with Nd1^T representing
93 approximately 90% of all cells as assessed by using double labelled oligonucleotide probes and
94 fluorescence *in situ* hybridization (DOPE-FISH) and 4',6-diamidino-2-phenylindole (DAPI)
95 staining (18). Growth of Nd1^T was also assessed by quantification of ammonia monooxygenase
96 sub-unit A (*amoA*) gene abundance (18). A pure culture was obtained by filtering out larger
97 contaminating bacteria (pore size 0.45 µm) but growth was initially only possible following
98 supplementation of medium with 80 mg l⁻¹ casamino acids or with filter-sterilised (pore size
99 0.22 µm) spent medium from the mixed enrichment culture previously incubated for 2 days.
100 After a further 10 subcultures, however, autotrophic growth in mineral salts medium containing
101 ammonium was achieved without supplementation with organic carbon (21). Purity of cultures
102 was confirmed by plating on 10% Tryptone Soya Agar plates for growing potential
103 heterotrophic contaminants, DOPE-FISH fluorescence microscopy for bacterial and archaeal
104 cells and a lack of PCR amplification of bacterial 16S rRNA genes.

105 A similar approach was used to enrich strain Nd2^T, but with incubation at 37°C and replacement
106 of streptomycin with 50 mg l⁻¹ kanamycin. A pure culture of this strain was obtained after
107 several subcultures without the need for filtration or supplementation with organic carbon (21).
108 Both species are routinely cultured by 2% (v/v) inoculation of late exponential phase cultures
109 into mineral salts medium containing 500 µM ammonium chloride adjusted to pH 5.3 with the
110 addition of 5 mM MES (2-(N-morpholino)ethanesulfonic acid) buffer. Cultures are incubated
111 at 25°C (strain Nd1^T) and 37°C (strain Nd2^T).

112 **Phylogeny**

113 The two strains possess a close phylogenetic relationship with 99.2% 16S rRNA identity. These
114 sequences were placed within the well-supported monophyletic Group I.1a-associated cluster
115 (candidate order *Nitrosotaleales*) and share a maximum of 90% 16S rRNA gene identity with
116 organisms placed within the Group I.1a cluster (order *Nitrosopumilales*) (Fig. 1a). The 16S
117 rRNA gene phylogeny was also congruent with phylogenomic analysis based on concatenated
118 single copy genes (Fig. 1b). The strains shared 95.5% *amoA* gene sequence identity and
119 were placed within the C14 (24) and NT-α-1 (25) *amoA* gene-defined lineages. Despite the
120 high identity of the marker gene sequences, the average nucleotide identity (ANI) between
121 Nd1^T, Nd2^T and the *Nitrosotalea* strain *Ca. Nitrosotalea okcheonensis* (26) all ranged between
122 79-83% (27), which is considerably below the species threshold of 95% (28). All three strains
123 (including *Ca. Nitrosotalea okcheonensis*) have an ANI lower than 70% with representatives
124 of the order *Nitrosopumilales*, which is the minimum level of identity between 11
125 representatives of the *Nitrosopumilales* (27). We therefore propose that strains Nd1^T and Nd2^T
126 be assigned to two different species as *Nitrosotalea devaniterrae* sp. nov. (formally described
127 as ‘*Ca. Nitrosotalea devanaterre*’ (18)) and *Nitrosotalea sinensis* sp. nov., respectively. We
128 also propose *Nitrosotalea* as the type genus within the family *Nitrosotaleaceae* fam. nov. (29)
129 and order *Nitrosotaleales* ord. nov. within the class *Nitrososphaeria* (30).

130 **Genome features**

131 The genomes of Nd1^T and Nd2^T were 1.81 and 1.60 Mb in length, which is comparable to type
132 strains from the order *Nitrosopumilales*, but smaller than soil-derived isolates from the order
133 *Nitrososphaerales* which are >2.5 Mb in size. The G+C mol% content of both genomes was
134 37% and slightly higher than seven type strains of the *Nitrosopumilales* (33-34%) of both
135 marine and soil origin but lower than *Nitrososphaera viennensis* EN76^T (52.7%) (Table 1).
136 Initial characterisation of the *N. devaniterrae* genome is described in Lehtovirta-Morley *et al.*
137 (31), with subsequent comparative analysis by Herbold *et al.* (27) of genomes of strains Nd1^T
138 and Nd2^T, the enrichment culture *Ca. Nitrosotalea okcheonensis* and a *Nitrosotalea*
139 metagenome-assembled genome (SbT1) with 19 other *Nitrososphaerota*. This analysis
140 identified a nitrososphaerotol core genome of 743 gene families, of which 103 formed a
141 *Nitrosotalea* core genome. This core is smaller than that of *Nitrososphaera* but larger than
142 those of other nitrososphaerotol genera. Within this core genome, 46 gene families were
143 homologous to genes in other *Nitrososphaerota* and there was evidence that 8 gene families
144 had been recruited by horizontal gene transfer from non-*Nitrososphaerota*, mainly acidophilic
145 organisms, suggesting their potential roles in acidophily adaptation.

146 **Phenotypic characterisation**

147 Characterisation of the initial enrichment culture of *N. devaniterrae* is described in Lehtovirta-
148 Morley *et al.* (18) and characterisation of pure cultures of both species is described in
149 Lehtovirta-Morley *et al.* (21). Cells of strains Nd1^T and Nd2^T are morphologically
150 indistinguishable under light or scanning electron microscopy and are rod-shaped (Fig. 2).
151 Cells have a slightly angular appearance, typical of AOA within the order *Nitrosopumilales*
152 (32), but distinct from the cocci or irregular cocci of the order *Nitrososphaerales* (33,34) and
153 *Ca. Nitrosocaldales* (35,36). The cells have electron-dense poles at which ribosomes are
154 concentrated (18) and have apparent intracellular particles and inclusion bodies the function of

155 which is unknown (Fig. 2c,d). Pure cultures of both species grow on inorganic, mineral salts
156 medium containing ammonium, obtaining cell carbon by CO₂ fixation and energy through
157 oxidation of ammonia to nitrite. Both are obligate acidophiles, with similar pH ranges for
158 growth, similar pH optima and no growth above pH 6.1. Maximum specific growth rates are
159 similar to (strain Nd2^T) or slightly lower than (strain Nd1^T) those of other *Nitrososphaerota*.
160 Cell yields are similar to those of other *Nitrososphaerota*, but cell-specific activities are lower
161 (21), and Nd1^T growth is inhibited at much lower concentrations of nitrite compared to
162 neutrophilic type strains (Table 1), likely due to the higher proportion of nitrite converted to
163 nitrous acid at low pH.

164 Membrane lipid compositions of strain Nd1^T and other AOA indicate that there is a
165 characteristic lipid profile specific to *Nitrososphaerota* that is distinct from that found in other
166 major archaeal phyla *Crenarchaeota* and *Euryarchaeota*. The cell membranes of all
167 *Nitrososphaerota* investigated to date contain a relatively complex pattern of core lipids, with
168 glycerol dibiphytanyl glycerol tetraethers (GDGT), glycerol diphytanyl diethers (archaeols,
169 AR), hydroxylated GDGTs (OH-GDGTs) and glycerol trialkyl glycerol tetraethers (GTGTs),
170 with intact polar lipids (IPL) consisting of monoglycosyl, diglycosyl, phosphohexose and
171 hexose-phosphohexose headgroups (37). GDGTs and GTGTs are found in all three archaeal
172 phyla, but the GDGT lipid crenarchaeol and methoxy archaeol (MeO-AR) are exclusive to
173 *Nitrososphaerota* (37). The relative abundance of MeO-AR in strain Nd1^T is highest (20.9%)
174 of all AOA strains investigated (31,37). The composition of core lipids is consistent with 16S
175 rRNA-gene phylogeny, providing both a basis for chemotaxonomy and potential links to
176 phenotype, adaptation and niche specialisation. There are also some correlations between IPL
177 content and habitat, with similarities between IPLs in strain Nd1^T, other terrestrial mesophiles
178 and terrestrial thermophiles. These results provide good evidence for phylogenetic
179 relationships between lipid composition and archaeal phyla. However, the importance of lipid

180 composition in *Nitrosotalea* species, its correlation with phylogeny and robust links between
181 phenotype and ecological distributions require analysis of more isolates.

182 A number of nitrification inhibitors have been developed for physiological studies but also for
183 commercial use to reduce loss of ammonia-based fertilisers in agricultural soils (38). Although
184 originally designed to target AOB, these inhibitors are generally similarly effective against
185 AOA. The sensitivity of strain Nd1^T to three such inhibitors, allylthiourea (ATU),
186 dicyandiamide (DCD) and nitrapyrin, has been investigated by Lehtovirta-Morley *et al.* (39)
187 at concentrations that lead to inhibition of AOB. Inhibition of ammonia oxidation by strain
188 Nd1^T in liquid batch culture by DCD and nitrapyrin is similar to that typically observed in AOB
189 (38), with complete inhibition at concentrations of 5 mM and 10 µM, respectively. ATU,
190 however, is less effective, with only 85% inhibition at a concentration of 100 µM, which would
191 typically inhibit AOB activity. Nitrapyrin has a similar inhibitory effect on growth as for AOB.
192 ATU and DCD reduce, but do not stop increases in cell abundance, even when ammonia
193 oxidation is significantly inhibited, possibly due to utilisation of storage compounds. These
194 results are consistent with other reports of reduced sensitivity of AOA to ATU (40,41,42).

195 There is currently no direct evidence of mixotrophic growth of AOA, including *Nitrosotalea*.
196 Early suggestions of mixotrophy arose through stimulation of growth of another soil AOA, *N.*
197 *viennensis* EN76^T, by pyruvate (33) and of *N. maritimus* SCM1^T by pyruvate and α -
198 ketoglutarate (32). There is now strong evidence that the α -keto acids pyruvate and
199 oxaloacetate are not assimilated but stimulate growth by detoxifying intracellular H₂O₂
200 produced during ammonia oxidation (43). Intermediates of the tricarboxylic acid cycle have
201 differing effects on growth of strain Nd1^T and strain Nd2^T (21) and, in some cases, effects of
202 individual organic acids on specific growth rate and yield differ within the same species.
203 Pyruvate reduces the growth yield of both species and reduces the maximum specific growth
204 rate of strain Nd1^T, but not strain Nd2^T. In general, strain Nd1^T is more sensitive to inhibition

205 by organic acids than strain Nd2^T. Both species are inhibited by nitrous acid and strain Nd1^T is
206 inhibited at ammonia concentrations >50 mM.

207 **Ecology**

208 The major ecological significance of the *Nitrosotalea* genus is its contribution to explaining
209 the paradoxical occurrence of high rates of ammonia oxidation and nitrification in acidic soils,
210 which comprise approximately 30% of soil globally. There is evidence of adaptation of some
211 AOB phylogenetic lineages to acidic conditions (44), the growth range or activity of cultivated
212 neutrophilic AOB can extend down to pH 5.0 such as for *Nitrosospira* sp. AF (13) and
213 *Nitrosospira lacus* (14) and AOB can be isolated from acid soils (45). However, with
214 exception of the gammaproteobacterial genera *Candidatus Nitrosoglobus* (15,46) and
215 *Candidatus Nitrosacidococcus* (47), no cultivated AOB grow optimally on ammonium at pH
216 values below 7 or are obligately acidophilic. This was believed to be through increasing
217 ionisation of NH₃ (the proposed substrate for ammonia monooxygenase) to NH₄⁺ with
218 decreasing pH, thus reducing available substrate concentration. Consistent with the substrate
219 limitation at low pH, strains Nd1^T and Nd2^T have remarkably low half-saturation constants and
220 high affinities for ammonia ($K_{m(app)} = \sim 0.6\text{--}2.8$ nM NH₃) (48). Molecular data provided
221 evidence that AOA were more abundant and active than AOB in acidic soils (16,17) and the
222 potential existence of acidophilic AOA. This was confirmed by cultivation of the acidophile
223 strain Nd1^T (18), subsequent isolation and enrichment of other acidophilic *Nitrosotalea* strains
224 (21,26) and demonstration of autotrophic growth of *Nitrosotalea* phylotypes in acid soil
225 (17,18). This therefore provides the most parsimonious explanation for this paradox, with
226 consequences for mitigation of fertiliser loss and nitrous oxide emissions in acid soils (49).

227 **Description of *Nitrosotalea* gen. nov.**

228 *Nitrosotalea* (Ni.tro.so.ta'le.a. N.L. masc. adj. *nitrosus*, nitrous; L. fem. n. *talea*, slender rod;
229 N.L. fem. n. *Nitrosotalea*, a slender rod producing nitrite). Aerobic. Chemolithoautotrophic
230 growth by ammonia oxidation to nitrite. Organic carbon obtained by CO₂ fixation. Mesophilic,
231 with optimal temperatures between 25°C and 37°C. Acidophilic, growing in the pH range 4.2
232 – 6.1. Free living in terrestrial ecosystems, particularly acidic soils. *Nitrosotalea* strains possess
233 >99% 16S rRNA gene identity with each other and 89-90% 16S rRNA gene identity within
234 representatives of the order *Nitrosopumilales*. G+C content is low, as is typical for many AOA,
235 ranging from 37.1-37.4 mol%. Currently comprises two isolates in pure culture, the type
236 species *Nitrosotalea devaniterrae*, *Nitrosotalea sinensis* and an enrichment culture, *Ca.*
237 *Nitrosotalea okcheonensis* CS (27).

238 **Description of *Nitrosotalea devaniterrae* sp. nov.**

239 *Nitrosotalea devaniterrae* sp. nov. (de.va.ni.ter'rae. L. fem. n. *Devana* the Roman name for
240 Aberdeen; L. fem. n. *terra* soil; N.L. gen. n. *devaniterrae* of soil from Aberdeen, describing its
241 habitat of isolation).

242 Displays the following properties in addition to those described for the genus. Rod-shaped cells
243 0.33 µm × 0.89 µm. Maximum specific growth rate 0.011 h⁻¹, cell yield 6.45 × 10⁴ cells (µM
244 NH₄⁺)⁻¹ and specific cell activity 0.072 fmol NO₂⁻ cell⁻¹ h⁻¹ (equivalent to approx. 7 µmol (NH₄⁺
245 mg protein⁻¹ h⁻¹). Growth in the pH range 4.2 – 5.6, optimum pH 5.2 and temperature range
246 20°C - 30°C, optimum 25°C. Yield increased by oxaloacetate and decreased by pyruvate and
247 citrate. Specific growth rate reduced by pyruvate, citrate, α-ketoglutarate, succinate, fumarate,
248 and malate. Growth inhibited by 0.91 – 3.5 µM HNO₂.

249 The genome is a single circular 1.81-Mb chromosome with 2,103 coding sequences and a G+C
250 content of 37.1 mol% and an ANI of ≤83% with other cultivated representatives of the

251 *Nitrosotalea*. NCBI accession numbers for complete genome and 16S rRNA gene sequences
252 are PRJEB10948 and JN227488, respectively.

253 The type strain is *Nitrosotalea devaniterrae* Nd1^T (=NCIMB 15248^T=DSM 110862^T), which
254 was isolated from an acidic (pH 4.5) agricultural soil located near Aberdeen (Scotland, UK).

255 **Description of *Nitrosotalea sinensis* sp. nov.**

256 *Nitrosotalea sinensis* sp. nov. (si.nen'sis. N.L. fem. adj. *sinensis*, from China, describing its
257 habitat of isolation).

258 Displays the following properties in addition to those described for the genus. Rod-shaped cells
259 0.22 µm × 0.76 µm. Maximum specific growth rate 0.025 h⁻¹, cell yield 7 × 10⁴ cells (µM
260 NH₄⁺ and specific cell activity 0.065 fmol NO₂⁻ cell⁻¹ h⁻¹ (equivalent to approx. 6.3 µmol NH₄⁺
261 mg protein⁻¹ h⁻¹). Growth in the pH range 4.0 – 6.1, optimum pH 5.2, and temperature range
262 20°C – 42°C, optimum 35°C. Yield simulated by oxaloacetate and inhibited by pyruvate and
263 fumarate. Growth inhibited by 1.61 - 5.7 µM HNO₂.

264 The genome is a 1.60-Mb chromosome with 1,888 coding sequences and a G+C content of
265 37.4 mol% and an ANI of ≤79% with other cultivated representatives of the *Nitrosotalea*.
266 NCBI accession numbers for draft genome and 16S rRNA gene sequences are
267 GCF_900143675 and KJ540205, respectively.

268 The type strain is *Nitrosotalea sinensis* Nd2^T (=NCIMB 15249^T=DSM 110863^T), which was
269 isolated from an acidic sulphate paddy soil (pH 4.7) sampled in Taishan County, Guangdong
270 Province in Southern China.

271 **Description of *Nitrosotaleaceae* fam. nov.**

272 *Nitrosotaleaceae* (Ni.tro.so.ta.le.a.ce'ae. N.L. fem. n. *Nitrosotalea*, type genus of the family; -
273 *aceae*, ending to denote a family; N.L. fem. pl. n. *Nitrosotaleaceae*, the family of the genus
274 *Nitrosotalea*).

275 Mesophilic, rod-shaped cells. Obligately acidophilic and obligately aerobic,
276 Chemolithoautotrophic, fixing carbon dioxide and oxidising ammonia to nitrite. Phylogeny of
277 both *amoA* and 16S rRNA genes provides support for a distinct branch within the phylum
278 *Nitrososphaerota*. Currently comprises a single genus, *Nitrosotalea*. Further details of the
279 family are provided in the description of the order *Nitrosotaleales*.

280 **Description of *Nitrosotaleales* ord. nov.**

281 *Nitrosotaleales* (Ni.tro.so.ta.le.a'les. N.L. fem. n. *Nitrosotalea*, type genus of the order; -*ales*,
282 ending to denote an order; N.L. fem. pl. n. *Nitrosotaleales*, the order of the genus *Nitrosotalea*).

283 Small rods, 0.5 - 1 µm in length with a slight angular appearance and electron-dense poles.
284 Obligate aerobes and obligate acidophiles, growing in the pH range 4.0 – 6.1 and the
285 temperature range 20 – 40°C. Chemolithotrophs that fix carbon dioxide and gain energy by
286 oxidising ammonia to nitrite. The order *Nitrosotaleales* refers to the Group I.1a-associated
287 *Nitrososphaerota*, which is phylogenetically distinct from the orders *Nitrosopumilales* (which
288 refers to Group I.1a and is represented by *Nitrosopumilus maritimus* SCM1^T) (32),
289 *Nitrososphaerales* (which refers to Group I.1b and is represented by *Nitrososphaera viennensis*
290 EN76^T) (30) and 'Candidatus Nitrosocaldales' (50) (which refers to the ThAOA group and is
291 represented by 'Candidatus Nitrosocaldus yellowstonensis' HL72) (51) within the class
292 *Nitrososphaeria*. Found in soil, particularly acidic soil. This order contains the family
293 *Nitrosotaleaceae*. The type genus is *Nitrosotalea*.

294 **Conflicts of interest**

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

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303

304

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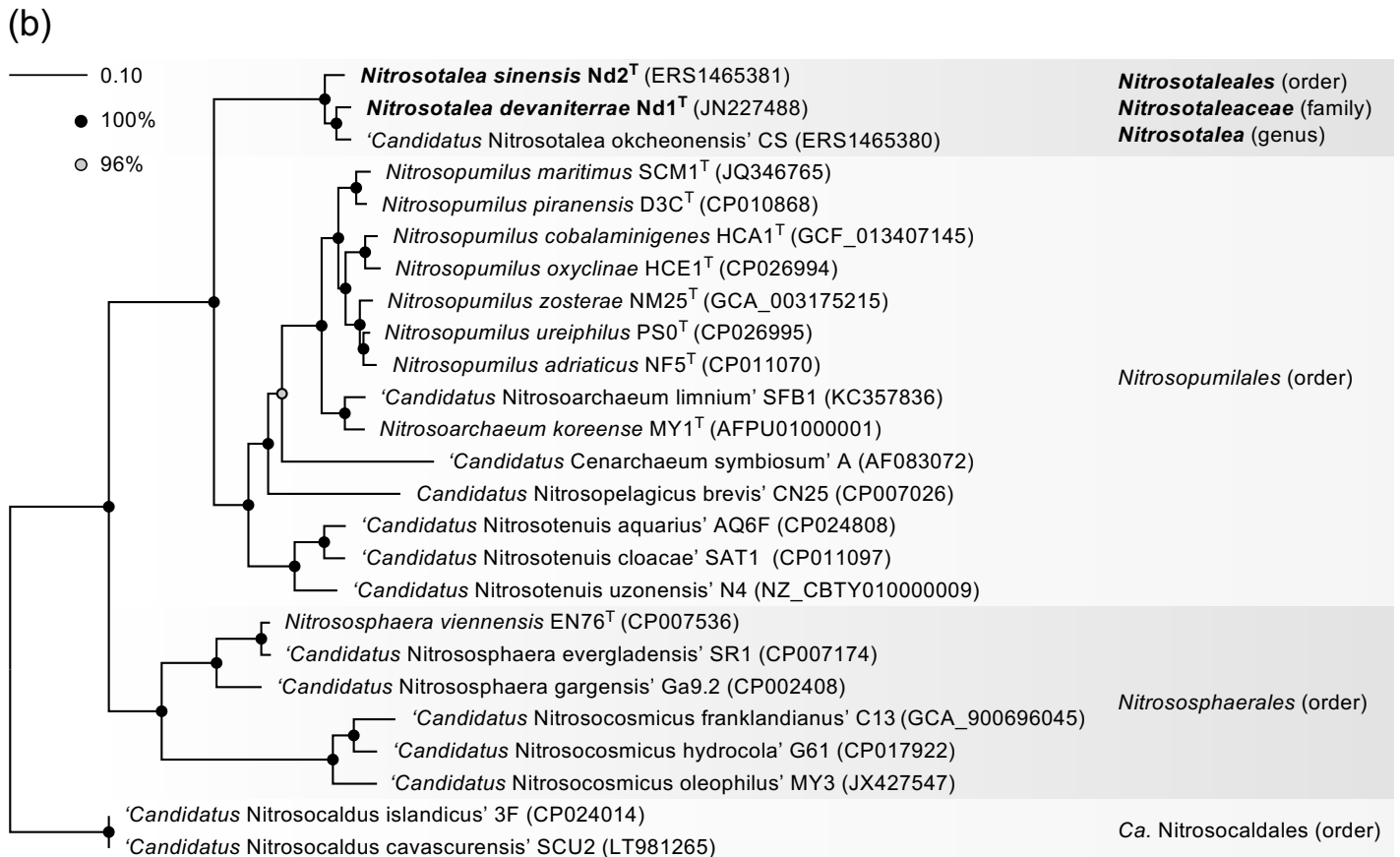
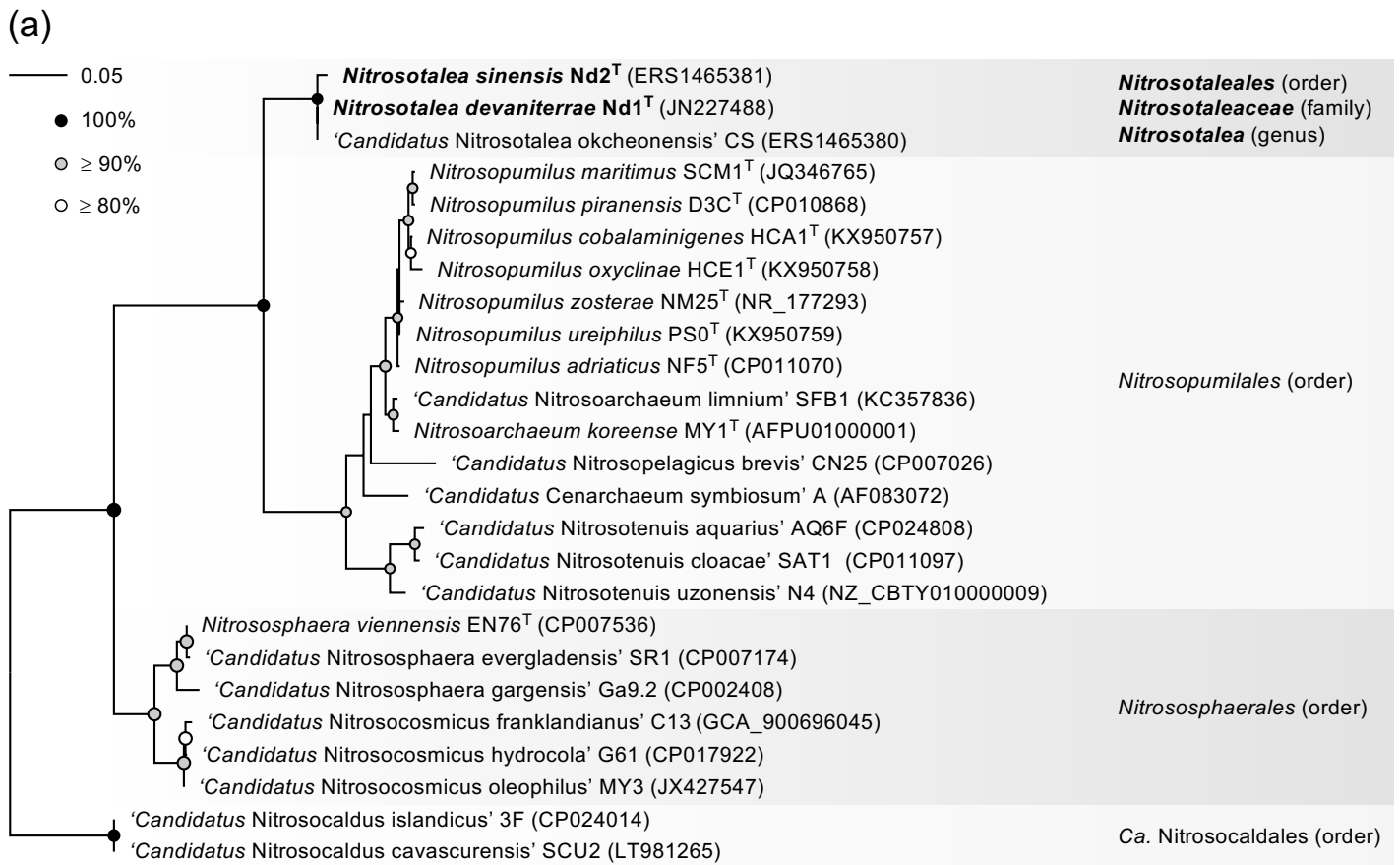
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484

485 **Figure legends**

486 **Figure 1.** Phylogenetic relationship of strains Nd1^T and Nd2^T with other candidate or validated
487 strains of the class *Nitrososphaeria*. Maximum likelihood phylogenies were generated using
488 PhyML (52) with automatic model selection. (a) Phylogenetic analysis of 16S rRNA genes
489 using 1,423 unambiguously aligned positions using the general time reversible (GTR)
490 substitution model with estimated gamma and invariant distribution of sites. The scale bar
491 represents an estimated 0.05 changes per nucleotide position and circles at nodes describe
492 bootstrap support (1,000 replicates). (b) Phylogenetic analysis of concatenated protein
493 sequences (7,951 unambiguously aligned amino acid positions) inferred from 45 single copy
494 genes generated using GToTree (53) and determined using the LG substitution model with
495 freerate variation across sites. The scale bar represents an estimated 0.1 changes per nucleotide
496 position and circles at nodes describe bootstrap support (100 replicates).

497 **Figure 2.** Scanning electron micrographs (a, b) and transmission and electron micrographs (c,
498 d) of strains Nd1^T (a, c) and Nd2^T (b, d). Scale bars are 1 μm (a, b) and 0.5 μm (c, d) and arrows
499 indicate subcellular particles. (From (21), with permission.)



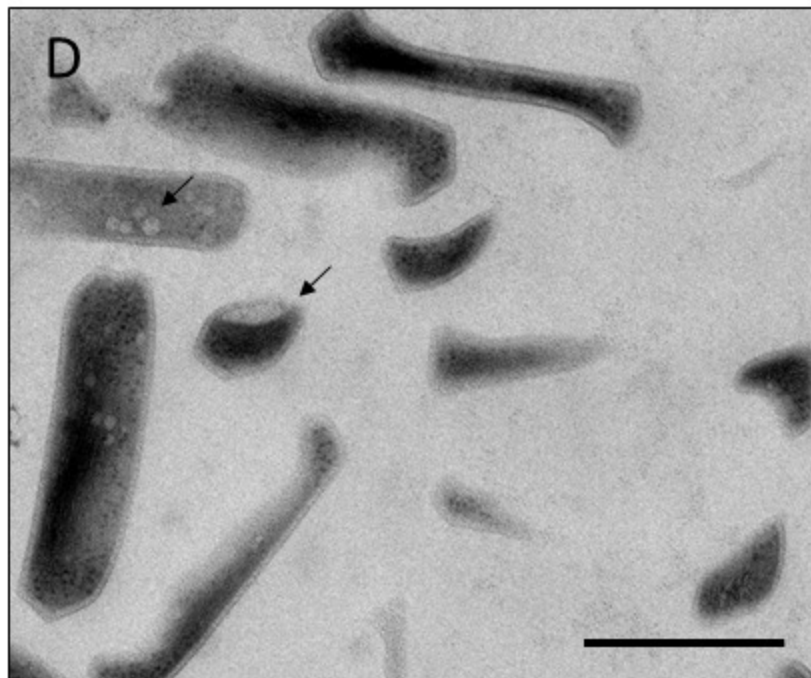
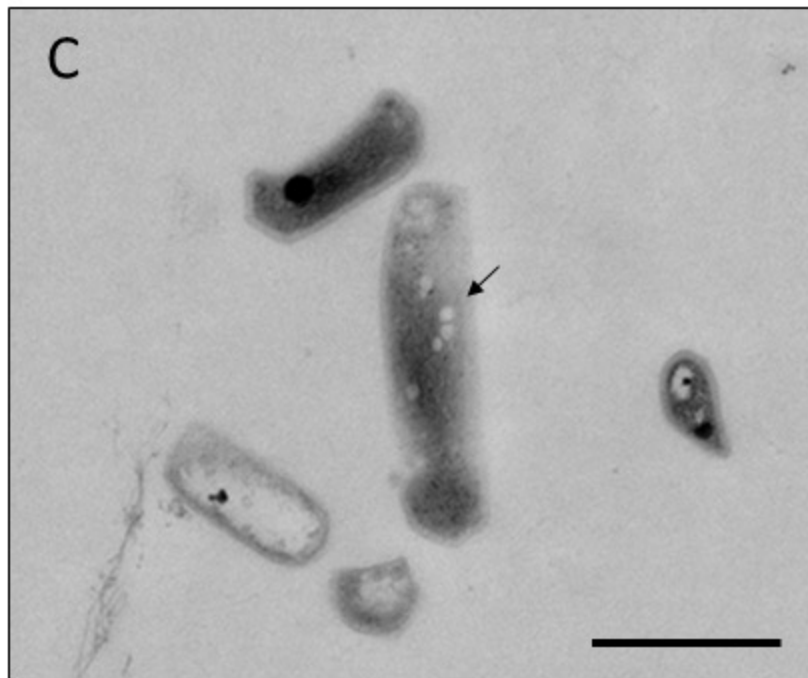
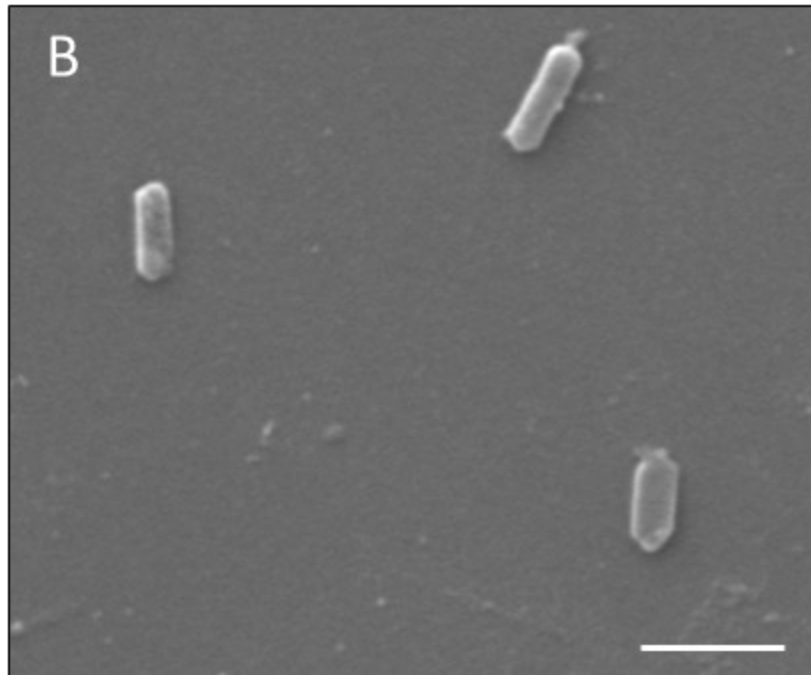
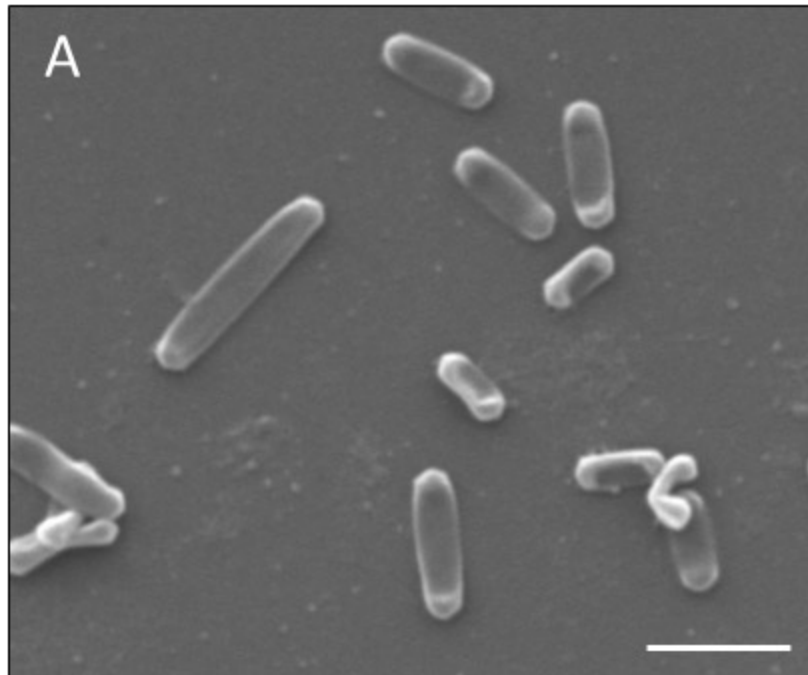


Table 1. Characteristics of ND1^T and ND2^T compared to validated strains within the class *Nitrososphaeria*. Data for *Nitrosopumilus maritimus* SCM1^T, *Nitrosopumilus cobalaminigenes* HCA1^T, *Nitrosopumilus oxyclinae* HCE1^T and *Nitrosopumilus ureiphilus* PS0^T were previously reported by Qin et al. [32], *Nitrosopumilus piranensis* DC3^T and *Nitrosopumilus adriaticus* NF5^T by Bayer et al. [54], *Nitrosopumilus zosterae* NM25^T by Nakagawa et al. [55], *Nitrosarchaeum koreense* MY1^T by Jung et al. [42] and *Nitrososphaera viennensis* EN76^T by Stieglmeier et al. [30]. ND, no data; -, negative; +, positive.

Characteristic	Nd1 ^T	Nd2 ^T	D3C ^T	HCA1 ^T	HCE1 ^T	NF5 ^T	NM25 ^T	PS0 ^T	SCM1 ^T	MY1 ^T	EN76 ^T
Validated genus	<i>Nitrosotalea</i>	<i>Nitrosotalea</i>	<i>Nitrosopumilus</i>	<i>Nitrosopumilus</i>	<i>Nitrosopumilus</i>	<i>Nitrosopumilus</i>	<i>Nitrosopumilus</i>	<i>Nitrosopumilus</i>	<i>Nitrosopumilus</i>	<i>Nitrosoarchaeum</i>	<i>Nitrososphaera</i>
Habitat source	Soil (pH 4.5)	Soil (pH 4.7)	Seawater	Seawater	Seawater	Seawater	Sediment	Sediment	Marine aquarium	Soil (pH 5.6)	Soil (pH 8.0)
Growth temperature (°C)											
Range	20-30	20-42	15-37	10-30	04-30	15-34	20-37	10-30	16-35	15-30	28-47
Optimum	25	35	32	25	25	30-32	30	26	32	25	42
Growth pH											
Range	4-5.5	4-6.1	6.8-8.0	6.8-8.1	6.4-7.8	6.8-8.0	6.1-7.7	5.9-8.1	6.8-8.1	6.0-8.0	6.0-8.0
Optimum	5.2	5.2	7.1-7.3	7.3	7.3	7.1	7.1	6.8	7.3	7	7.5
Max. ammonia oxidation (fmol cell ⁻¹ day ⁻¹)	1.7	1.5	10.9	6	5.8	9.6	ND	2.9	12.7	12.7	ND
Min. generation time (h)	57	28	27	30	33	34	28	28	45	19	28
Ammonium tolerance (mM)	>10 mM	ND	30	10	1	25	ND	20	12.7	10	15
Nitrite tolerance (mM)	<0.1	ND	10	ND	ND	15	ND	ND	2	5	10
Use of urea	-	-	+	-	-	-	-	+	-	-	+
Genome size (Mb)	1.81	1.60	1.71	1.56	1.59	1.8	1.76	2.17	1.65	1.61	2.52
DNA G+C content (mol%)	37.1	37.4	33.8	33.0	33.1	33.4	33.8	33.4	34.2	32.7	52.7