

Isolation and characterization of *Neisseria musculi* sp. nov., from the wild house mouse

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Members of the genus *Neisseria* have been isolated from or detected in a wide range of animals, from non-human primates and felids to a rodent, the guinea pig. By means of selective culture, biochemical testing, Gram staining and PCR screening for the *Neisseria*-specific internal transcribed spacer region of the rRNA operon, we isolated four strains of the genus *Neisseria* from the oral cavity of the wild house mouse, *Mus musculus* subsp. *domesticus*. The isolates are highly related and form a separate clade in the genus, as judged by tree analyses using either multi-locus sequence typing of ribosomal genes or core genes. One isolate, provisionally named *Neisseria musculi* sp. nov. (type strain AP2031^T=DSM 101846^T=CCUG 68283^T=LMG 29261^T), was studied further. Strain AP2031^T/*N. musculi* grew well *in vitro*. It was naturally competent, taking up DNA in a DNA uptake sequence and *pilT*-dependent manner, and was amenable to genetic manipulation. These and other genomic attributes of *N. musculi* sp. nov. make it an ideal candidate for use in developing a mouse model for studying *Neisseria*–host interactions.

The family *Neisseriaceae*, a large family of Gram-negative bacteria, are most well known for two members that cause

diseases of importance to human health, *Neisseria meningitidis* and *Neisseria gonorrhoeae*. The other members of the family are commensals of humans and other animals. *N. meningitidis* and *N. gonorrhoeae* also exhibit commensal-like behaviour in that they establish asymptomatic infections in man at high frequency (Caugant & Maiden, 2009; Read, 2014; Turner *et al.*, 2002). This behaviour may be due to traits they inherited from their commensal forebears as they evolved a more pathogenic lifestyle (Marri *et al.*, 2010).

At least ten commensal species of the genus *Neisseria* are known to colonize humans, and an equal number or more have been isolated from, or detected in, a wide range of animals (Bennett *et al.*, 2014a; Liu *et al.*, 2015). Commensal species of the genus *Neisseria* have been isolated from the oropharynx and urogenital tract of man, the oral cavity and throat of guinea pigs, the liver and faeces of ducks, the oral cavity and dental plaque of cows, the upper respiratory tract and lung of dogs, and the oral cavity of a rhesus macaque (see Table 2 in Liu *et al.*, 2015; Barrett & Sneath, 1994; Bennett *et al.*, 2014a; Murphy *et al.*, 2005; Weyand *et al.*, 2013).

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Abbreviations: ANI, average nucleotide identity; DUS, DNA uptake sequence; ITS, internal transcribed spacer; MLST, multilocus sequence typing; cgMLST, MLST of core genes; rMLST, MLST of ribosomal genes; SmR, streptomycin resistant; Tfp, Type IV pili.

The GenBank/EMBL/DDBJ accession numbers for the partial *rpI* gene sequences of strains AP2031^T, AP2104, AP2105 and AP2119 are KT997727, KT997728, KT997729 and KT997730, respectively. The European Nucleotide Archive run identifier for the AP2031^T draft genome is ERR1121178. AP2031^T genome data has also been deposited in PubMLST databases and can be found at: www.pubmlst.org/neisseria/ or <http://pubmlst.org/mlst/> (ID 29520).

Five supplementary tables and four supplementary figures are available with the online Supplementary Material.

Many aspects of *Neisseria*–host interactions can be more readily dissected using a small animal model, for example, by pairing the genetically tractable mouse, *Mus musculus*, with a species of the genus *Neisseria* that naturally colonizes the animal. We therefore sought to isolate a mouse-dwelling strain of the genus *Neisseria*. Through selective culturing, biochemical testing and genotyping, we obtained four isolates of the genus *Neisseria*, representing a novel species in the genus, from the oral cavity of wild mice (*Mus musculus* subsp. *domesticus*) trapped in two geographically distinct regions in North America. One isolate, AP2031^T, was chosen for further study. AP2031^T grew well *in vitro*. It was naturally competent; DNA transformation was greatly enhanced by the presence of the neisserial DNA uptake sequence (DUS) and by an intact *pilT* gene. We propose that strain AP2031^T be classified as the type strain of *Neisseria musculi* sp. nov.

Oral swabs were collected from 36 healthy wild mice (*M. musculus* subsp. *domesticus*) live-trapped in two distinct regions of North America (Table S1, available in the online Supplementary Material). A subset of these mice (TAS216–TAS220) was sampled immediately for members of the genus *Neisseria*, then humanely euthanized for use in another study. All other mice were brought back to the lab and housed individually or in breeding pairs in static microisolator cages, with food and water *ad libitum*. Cages were changed weekly or biweekly in a laminar flow hood following standard protocols to minimize cross-cage contamination. After initial breeding, all animals were housed individually or in same-sex pairs for at least 6 weeks to ensure eradication of mouse pathogens that pose a risk to animal care facilities. Serologically negative mice were made available for sampling organisms of the genus *Neisseria*. All collection and husbandry activities were conducted in accordance with University of Arizona IACUC policies.

Mice that had passed the quarantine period were sampled for members of the genus *Neisseria*. The oral cavity was swabbed using the BD BBL CultureSwab Plus Transport System (Fisher Scientific). To enrich for growth of bacteria of the genus *Neisseria*, swab suspensions in GCB medium base (Beckton Dickinson) were plated on GCB agar containing vancomycin (2 mg l⁻¹) and trimethoprim (3 mg l⁻¹), and the plates were incubated for 48 h at 37 °C, 5 % CO₂.

To identify and speciate members of the genus *Neisseria*, we proceeded as follows. A portion from each colony growing on GCB vancomycin/trimethoprim agar was used for oxidase testing as described by Cheesbrough (2006) using oxidase reagent (PML Microbiologicals). Oxidase⁺ colonies were Gram-stained, and the internal transcribed spacer (ITS) region in the rRNA operon of Gram⁻ colonies was amplified by colony PCR, using primers specific to sequences that are highly conserved among species of the genus *Neisseria* (see Table S2 for primers). ITS⁺ colonies were streak-purified, assigned a strain number, and stored at -80 °C in GCB+glycerol (20 %, v/v). We identified four isolates that were oxidase⁺, Gram⁻ and *Neisseria* ITS⁺. Two of these isolates, AP2031^T and AP2119, were from mice trapped in Tucson, Arizona, USA (TUSA8 and TAS218,

respectively), and two isolates, AP2104 and AP2105, were from mice trapped in a haystack near Parkland, Alberta, Canada (TAS116 and TAS118, respectively) (Table S1). To further characterize these four isolates, their *rplF* sequences were determined and compared with those of other species of the genus *Neisseria*. A neighbour-joining tree was drawn with *rplF* sequences using MEGA5 (Tamura *et al.*, 2011). All three codon positions were included. Bootstrap support values (2000 replicates) greater than 70 % were added to nodes. Sequence comparisons of *rplF*, an approach that distinguishes closely related species in the genus *Neisseria* (Bennett *et al.*, 2014b), indicated the four isolates were tightly clustered in a separate clade (Fig. 1a). Surprisingly, they were not as related to *Neisseria animalis*, a guinea pig isolate, as they were to *Neisseria dentiae*, a cow dental plaque isolate (Berger, 1960; Sneath & Barrett, 1996).

We conducted average nucleotide identity (ANI) analysis for all four mouse isolates using BioEdit 7.2.5 (Hall, 1999). Only three polymorphic sites were detected in the *rplF* fragment sequenced. Since the four isolates share 99.4 % sequence identity, we conclude they belong to the same species, *N. musculi* sp. nov. This conclusion is based on the fact that *rplF* gene trees are more concordant with trees based on multi-locus sequence typing (MLST) of ribosomal genes (rMLST), the gold standard for identification of species of the genus *Neisseria*, whereas 16S rRNA gene trees lack such resolution (Bennett *et al.*, 2014b).

The genome of isolate AP2031^T, from Tucson mouse TUSA8, was sequenced at the Oxford Genomic Center, Wellcome Trust Centre for Human Genetics, University of Oxford, UK. Samples were quantified using PicoGreen (Invitrogen), and their integrity was assessed using 1 % E-Gel EX (Invitrogen). DNA was fragmented using the Episonic system and sized using a TapeStation D1200 system (Agilent/Lab901). Libraries were constructed with a NEBNext DNA Sample Prep Master Mix Set 1 kit (New England BioLabs), and adaptors were ligated using Illumina Adapters (Multiplexing Sample Preparation Oligonucleotide kit). Ligated libraries were size-selected using AMPure magnetic beads (Agencourt), PCR-enriched for 10 cycles as per Illumina recommendations, and purified with AMPure XP beads (Agencourt). Size distribution was determined using a TapeStation 1DK system (Agilent/Lab901), and multiplex pool concentrations were determined by PicoGreen (Invitrogen). Pooled libraries were quantified using the quantitative PCR Library Quantification kit and MX3005PTM instrument (Agilent). Sequencing was performed using the Illumina HiSeq 2000 system to generate 100 nt paired-end reads. Resulting short-read sequences were assembled *de novo* using the VelvetOptimiser algorithm as part of an in-house pipeline developed at the Department of Zoology, University of Oxford. The minimum output contig size was set to 200 bp with the scaffolding option switched off; all other program settings were left at default. No read trimming was performed. Draft genome sequences (ID 29520) were deposited in the *Neisseria* pubmlst.org database (www.pubmlst.org/neisseria). The

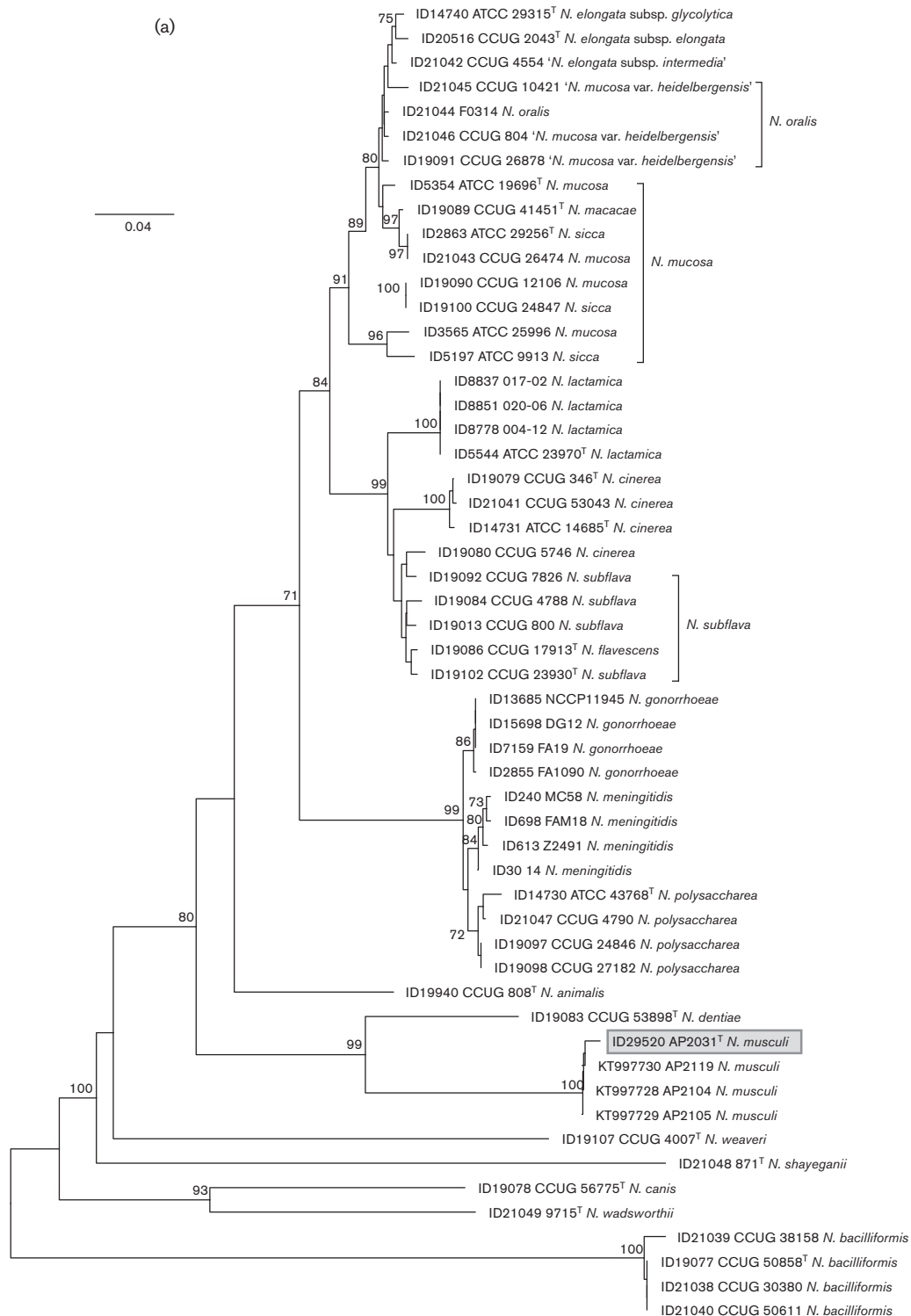
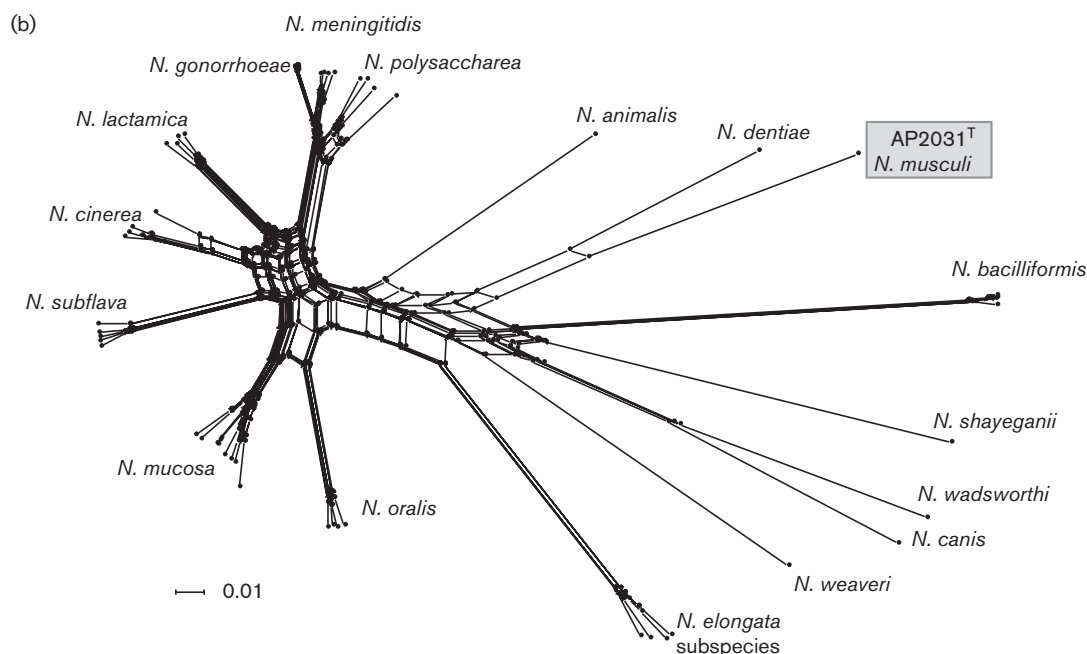


Fig. 1. (a) Evolutionary relationship of mouse *Neisseria* isolates AP2031^T (in grey box), AP2119, AP2104 and AP2105, to 50 isolates of the genus *Neisseria*, deduced from a neighbour-joining tree reconstructed from their *rplF* sequences. Bootstrap values $\geq 70\%$ are noted (2000 replications). Bar, 0.04 substitutions per nucleotide position. PubMLST ID or GenBank accession numbers precede the isolate and species notations. Taxa included for *rplF* comparisons were previously analysed by Bennett *et al.* (2014b). (b) Neighbour-net tree reconstructed from 51 concatenated ribosomal gene sequences, with AP2031^T highlighted in a grey box. Bar, 0.01 substitutions per nucleotide position.

Fig. 1. (cont.)



DNA G+C content of AP2031^T was 53.3 mol%, consistent with other species in the genus (Tonjum, 2005).

A detailed analysis of AP2031^T genome content will be presented in a future paper. Genomic data from AP2031^T was compared with a representative isolate dataset containing all known species of the genus *Neisseria* for which whole-genome sequencing data were available (Bennett *et al.*, 2012). MLST of 51 ribosomal genes (rMLST) (Fig. 1b) and 246 *Neisseria* core genes (cgMLST) (Fig. S1) was used to generate trees as described previously, enabling relationships between isolates to be determined and speciation (Bratcher *et al.*, 2014; Jolley *et al.*, 2012). Sequences were extracted from the bacterial isolate genome sequence database (BIGSdb) (Jolley & Maiden, 2010), aligned using MAFFT and imported into Splitstree thereby generating neighbour-net trees. Nucleotide sequences used for rMLST and cgMLST are listed in Table S3. These analyses confirmed the *rplF* findings on the evolutionary relationship of AP2031^T to other species of the genus *Neisseria*.

rplF, rMLST and cgMLST phylogenetic analysis revealed AP2031^T to be most closely related to *N. dentiae*. The *rplF* ANI between AP2031^T and *N. dentiae* was 85.1%. Other members of the genus, as expected, had lower ANI values (see Table S4). The ANI calculator at <http://enve-omics.ce.gatech.edu/ani/> was used to estimate the ANI between the AP2031^T and *N. dentiae* draft genome data sets. This tool calculates ANI as described by Goris *et al.* (2007). Consistent with the *rplF* analysis, an ANI value of 86.8% was obtained for the draft genomes of AP2031^T and *N. dentiae*. Given that ANI values lower than 95% delineate the genomes of

different species (Goris *et al.*, 2007), our findings further indicate AP2031^T and the other mouse *Neisseria* isolates represent a novel species in the genus *Neisseria*.

During passage on agar plates, *N. muscoli* sp. nov. (type strain AP2031^T) gave rise to colonies with smooth and rough phenotypes (Fig. S2). Images of colony morphology were acquired using a Cannon SLR digital camera attached to a Wild M7 zoom microscope (Heerbrugg, Switzerland) mounted on a Bausch and Lomb stage. All colonies of AP2031^T were circular and convex, and varied in opacity. Smooth colonies had shiny, unwrinkled surfaces, while rough colonies had bumpy, undulating surfaces. These phenotypes were interchangeable: smooth colony variants would give rise to rough colony variants and vice versa (data not shown). This process is reminiscent of colony phase variation observed for *N. gonorrhoeae*, which is caused by variable expression of surface components (Bhat *et al.*, 1991; Long *et al.*, 1998; Swanson & Barrera, 1983; Swanson *et al.*, 1971; Walstad *et al.*, 1977). The mechanism underlying AP2031^T colony phase variation is under investigation.

Smooth and rough variants formed biofilms when cultured on glass, and scanning electron microscopy revealed their distinct morphologies. Scanning electron microscopy was performed on AP2031^T cells as described by Higashi *et al.* (2011). Biofilms formed by the rough variant were taller and contained multiple layers of cells (Fig. 2). Rough variant cells are best described as diplococobacilli: short rods often found in pairs. The rods were approximately 0.4 µm in width and 0.5–0.8 µm in length (Fig. 2a, c, e, g). The

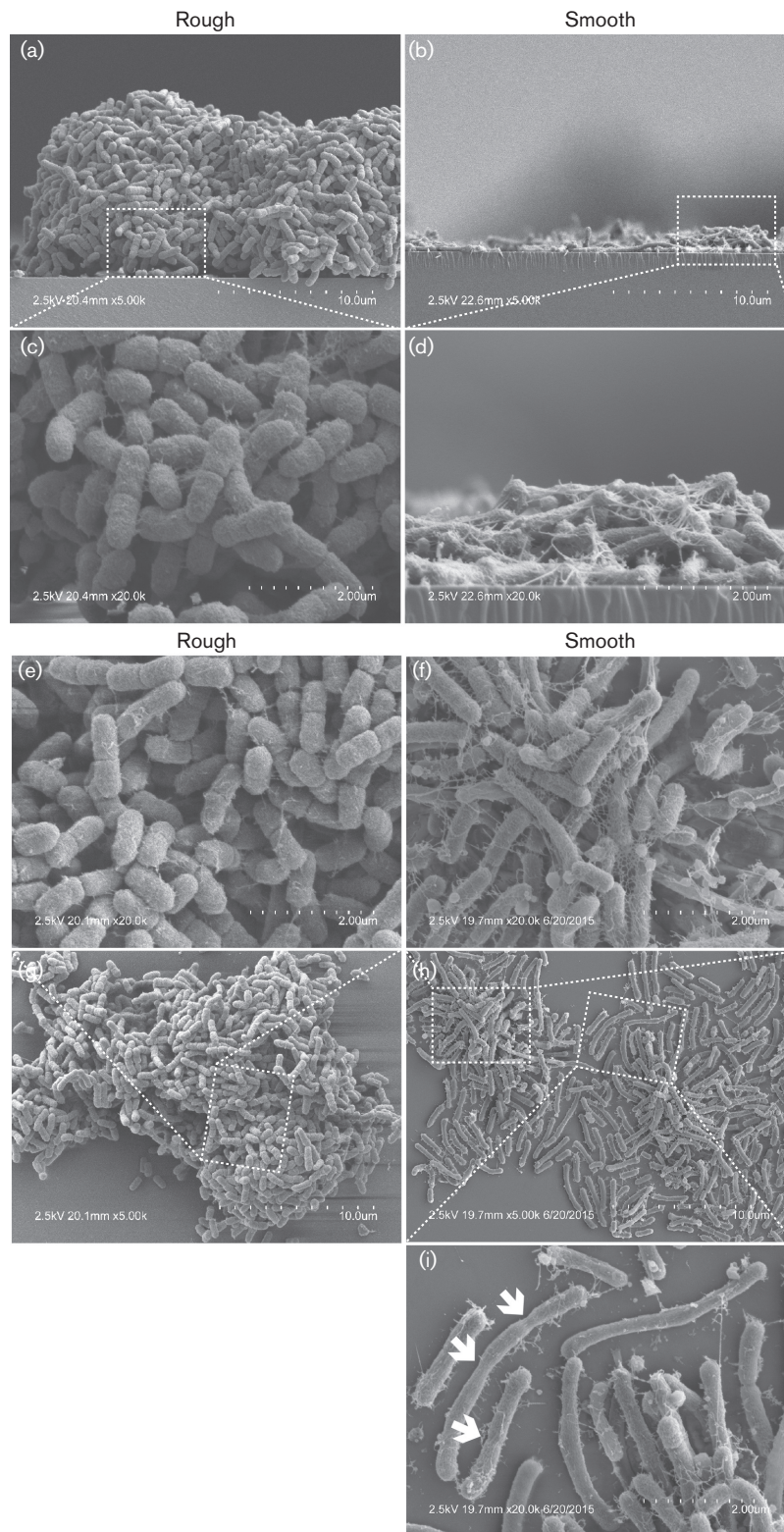


Fig. 2. Scanning electron microscopy images of cells of strain AP2031^T shown as side (a–d) and aerial (e–i) views. Images of rough (a, c, e, g) and smooth (b, d, f, h, i) colony variants after 3 h of growth on glass slides are shown. Boxed sections of images are shown at higher magnification in adjoining panels (c, d, e, f, i). White arrows in panel (i) indicate indentations along sheaths. Acquisition voltage, distance (mm) and scale bar are indicated.

shape of smooth variant cells was difficult to discern. Many appeared to be encased in sheaths of varying length with occasional indentations along their sides (Fig. 2b, d, f, h, i and S3). Several commensal species of the genus *Neisseria* are rod-shaped (e.g. *Neisseria elongata*, *Neisseria weaveri*, *Neisseria shayegani*, *Neisseria bacilliformis*, *Neisseria zoodegmatidis*, *Neisseria animaloris*) (Ganiere *et al.*, 1995; Liu *et al.*, 2015; Veyrier *et al.*, 2015), but there are no reports, to our knowledge, of sheath-like structures associated with cells of members of the genus *Neisseria*. The surfaces of smooth and rough variants were decorated with fibres, with rough variant fibres being shorter and less abundant. The nature of these fibres remains to be determined.

All species of the genus *Neisseria* studied to date are competent for DNA transformation. Playing important roles in this process are the Type IV pilus (Tfp) and the DUS, a 10 nt sequence, 5'-GCCGTCTGAA-3', that is highly enriched in neisserial genomes. Tfp-mediated DNA transformation involves, among other things, the binding of the DUS to Tfp-associated protein CompP, and PilT-driven retraction of the Tfp (Cehovin *et al.*, 2013; Hamilton & Dillard, 2006; Wolfgang *et al.*, 1998). Two DUS variants, distinguished by two bases immediately preceding the 10-mer, are abundant in neisserial genomes: 5'-agGCCGTCTGAA-3' and 5'-atGCCGTCTGAA-3'. agDUS is most abundant in human commensals *N. elongata*, *N. bacilliformis* and *Neisseria subflava*, and the dog commensal *N. weaveri* (Frye *et al.*, 2013; Mell & Redfield, 2014), while atDUS is most abundant in human commensals *Neisseria cinerea*, *Neisseria lactamica* and *Neisseria polysaccharea*, and the human pathogens *N. gonorrhoeae* and *N. meningitidis*.

To determine the number of copies of DUS in the AP2031^T genome, and to extract the DUS and surrounding sequences into a fasta file for constructing the WebLogo, Perl regular expression pattern matching in combination with the fuzznuc program from EMBOSS was used (Crooks *et al.*, 2004; Rice *et al.*, 2000). There are 3893 copies of the 10-mer DUS in AP2031^T, of which 2614 are agDUS and 119 are

atDUS (Fig. S4). AP2031^T has a complete set of genes for the biogenesis of the Tfp including *pilT*, which encodes the motor protein for the Tfp fibre (Table S5). The abundance of the neisserial DUS and the presence of Tfp genes strongly suggest that AP2031^T is naturally competent for DNA transformation.

To test the competence of AP2031^T, we determined the transformation frequency of the smooth variant in the presence of genomic DNA from AP2098, a naturally occurring streptomycin-resistant (Sm^R) variant. AP2098 was isolated by plating the original AP2031^T strain on GCB plates containing streptomycin (100 mg l⁻¹). Genomic DNA from AP2098 was used for transformation assays using previously described procedures (Weyand *et al.*, 2013). Transformation mixtures were plated on GCB agar with and without streptomycin (100 mg l⁻¹). Transformation frequency was expressed as the number of Sm^R c.f.u./total c.f.u. per µg of DNA. For some negative controls, DNA was incubated for 20 min with 2 units of DNaseI prior to transformation (New England Biolabs). The frequency was 1.7×10⁻⁴ (SEM±4.8×10⁻⁵) in the presence of DNA, and >3 logs lower in its absence or when the DNA was predigested with DNase (Table 1).

To determine the role of the DUS in AP2031^T transformation, cells were incubated with synthetic DNA amplified by PCR and encoding *rpsL* from AP2098 (Sm^R), with or without the 10-mer DUS 5'-GCCGTCTGAA-3' (see Table S2 for primers). DNA (1 µg) was used for transformation assays, and transformation frequencies were calculated as described above. The transformation frequency was nearly 3 logs higher when the transforming DNA contained the DUS (Table 1).

Finally, we determined the role of *pilT* in AP2031^T competence. Primers NP242F and NP242R (Table S2) containing flanking sequences for the AP2031^T*pilT* gene were used to amplify a kanamycin cassette from plasmid pNBNeiKan (synthesized by Genescript) for use in constructing gene deletions (Datsenko & Wanner, 2000). A DUS and an *Escherichia coli*

Table 1. Transformation frequency of mouse *Neisseria* strains

Recipient strain	DNA	Transformation frequency (×10 ⁻⁴)*
AP2031 ^T	AP2031-SmR gDNA	1.7±0.48
AP2031 ^T	AP2031-SmR gDNA + DNase	<0.00024±0.000071
AP2031 ^T	No DNA	<0.00034±0.00013
AP2031 ^T	<i>rpsL</i> ^{SmR} + DUS	0.43±0.4
AP2031 ^T	<i>rpsL</i> ^{SmR}	0.0016±0.00079
AP2031 ^T	No DNA	<0.00016±0.000031
AP2365 (AP2031-Rif ^R)	AP2031-SmR gDNA	1.249±0.55
AP2365 Δ <i>pilT</i> ::Kan	AP2031-SmR gDNA	<0.0015±0.00037
AP2365	No DNA	<0.00091±0.00023
AP2365 Δ <i>pilT</i> ::Kan	No DNA	<0.0019±0.00043

gDNA: genomic DNA; *rpsL*: PCR amplicon of *rpsL* locus with Sm^R mutation; Rif^R, rifampicin-resistant.

*Transformation frequencies are expressed as the number of Sm^R c.f.u./total c.f.u. Values are the mean from three independent experiments±SEM. '<', Transformation frequency below the limit of detection.

σ 70-dependent consensus promoter upstream of the cassette promote transformation and expression of the kanamycin resistance gene, respectively. The length of the *pilT* flanking sequences in the amplicon was extended by PCR using primers NP252F and NP252R. The amplification products were purified and used in spot or liquid transformations (Dillard, 2006) of AP2031^T to replace the *pilT* gene with a kanamycin resistance marker. Primers NP245F and NP245R were used to amplify the mutated region (Δ *pilT*::*kan*), and the amplification product was cloned into pGEMT (Promega). The resulting plasmid DNA was used for transformations aimed at replacing the *pilT* locus with Δ *pilT*::*kan* in a rifampicin-resistant strain background (see AP2365, Table 1). Transformants were selected and maintained on GCB containing kanamycin (50 mg l⁻¹). *pilT* mutants were confirmed by Sanger sequencing following PCR with primers NP245F and NP245R. The Δ *pilT* mutant transformed at a frequency approximately 3 logs lower than its wt parent (Table 1). Taken together, these results indicate AP2031^T is naturally competent, and DNA uptake involves the neisserial DUS and Tfp.

By means of selective culture, oxidase testing, Gram staining and PCR testing for the *Neisseria* ITS, we isolated four strains of the genus *Neisseria* from the oral cavity of healthy wild mice (*M. musculus* subsp. *domesticus*) trapped in Tucson, Arizona, USA, and Parkland, Alberta Province, Canada. *rplF* sequence comparisons indicate they are very closely related to each other and form a distinct clade in the genus *Neisseria*. Their closest phylogenetic neighbour is *N. dentiae*, a bovine isolate (Fig. 1) (Sneath & Barrett, 1996). This analysis is confirmed by trees of the Tucson mouse isolate, AP2031^T, based on MLST (Figs 1b and S1). We propose that the strains of the genus *Neisseria* isolated from mouse be classified as representatives of *N. musculi* sp. nov. (type strain AP2031^T). The rMLST approach unambiguously identifies members of the genus *Neisseria* (Bennett *et al.*, 2012). Our rMLST and core gene analyses indicate AP2031^T/*N. musculi* is a member of the genus *Neisseria*. This assignment is supported by the DUS sequence analysis of AP2031^T/*N. musculi* (Fig. S4).

AP2031^T/*N. musculi* sp. nov. is naturally competent; DNA transformation is greatly enhanced by the DUS and *pilT*, which encodes the Tfp motor protein (Table S5). Tfp is a hallmark of the genus *Neisseria*. Tfp consists of a fibre, composed mainly of pilin subunits, that extends from the cell into the extracellular milieu, and machinery for its assembly and many biological activities (Carbonnelle *et al.*, 2006; Giltner *et al.*, 2012). In addition to DNA transformation (Cehovin *et al.*, 2013; Wolfgang *et al.*, 1998), Tfp plays important roles in other aspects of biology of the genus *Neisseria*, including bacterial attachment, motility, micro-colony formation and signalling to the host cell (Eriksson *et al.*, 2012; Helaine *et al.*, 2005; Higashi *et al.*, 2007; Howie *et al.*, 2005; Kurre *et al.*, 2012; Lee *et al.*, 2005; Merz *et al.*, 1996, 1999, 2000; Nassif *et al.*, 1994; Pujol *et al.*, 1999; Winther-Larsen *et al.*, 2001; Wolfgang *et al.*, 1998). That AP2031^T/*N. musculi* harbours the genes necessary for the biosynthesis, assembly and anchoring of the Tfp fibre; for

the retraction and mechanotransductive properties of the fibre; and for DNA transformation (Table S5) strongly suggest that it expresses Tfp that functions similarly to those of other species in the genus.

Pilins in Tfp of pathogenic members of the genus *Neisseria* are decorated with mono-, di- or tri-saccharides, and/or phosphorylated residues (Aas *et al.*, 2006; Forest *et al.*, 1999; Hartley *et al.*, 2011; Naessan *et al.*, 2008). These post-translational modifications are thought to contribute to Tfp bundling, neisserial interactions with human cells and protection of the bacteria from immune defenses (Chamot-Rooke *et al.*, 2011; Jennings *et al.*, 2011; Marceau *et al.*, 1998). AP2031^T/*N. musculi* sp. nov. encodes enzymes for the biosynthesis and covalent linkage of the basal monosaccharide to pilin (*pglB*, *pglB2*, *pglC* and *pglD*), but lacks the genes that add additional sugars to the monosaccharide (*pglA* and *pglE*). This suggests its pilin may be decorated with monosaccharides. It should be noted that pilins in *N. elongata* Tfp are not glycosylated even though the commensal harbors *pgl* genes (Anonsen *et al.*, 2015). Whether this lack of glycosylation is due to control of gene expression is unclear. Finally, AP2031^T/*N. musculi* lacks the genes (*pptA* and *pptB*) that phosphorylate pilin (Anonsen *et al.*, 2012; Naessan *et al.*, 2008).

In summary, we have isolated a novel species in the genus *Neisseria* from the oral cavity of wild mice. We determined the ability of one of these isolates, type strain AP2031^T, to take up neisserial DNA, and sequenced its genome. AP2031^T had the following characteristics that support its inclusion in the genus *Neisseria*. Firstly, it stained Gram-negative. Secondly, it had oxidase activity. Thirdly, genus-specific primers successfully amplified the 16S ITS region. Fourthly, *rplF* and MLST phylogenic analysis determined that AP2031^T belongs in the genus and is most closely related to *N. dentiae*, an established species of the genus *Neisseria*. Fifthly, AP2031^T genome sequencing established that this isolate has over 3000 copies of the best characterized *Neisseria*-specific repetitive element, the DUS.

AP2031^T/*N. musculi* sp. nov. grows readily in the lab and is amenable to genetic manipulation. These qualities of AP2031^T, coupled with its genetic relatedness to other species of the genus *Neisseria*, make the isolate an excellent candidate for use in developing a mouse model for probing *Neisseria*-host interactions. Such a model will circumvent roadblocks imposed by host tropism, an issue that makes studying human-dwelling members of the genus *Neisseria* problematical. It will allow assessment of the role of the Type IV pilus in colonization by members of the genus *Neisseria*, and dissection of immune responses mounted by the host (the mouse) to the commensal species of the genus *Neisseria*.

Description of *Neisseria musculi* sp. nov.

Neisseria musculi (mus'cu.li. L. gen. n. *musculi* of a mouse).

Cells are diplococci (approx. 0.5 to 0.8 µm in length) Gram-stain-negative and oxidase-positive. Good growth

occurs on chocolate agar, TSA with 5% sheep blood and GCB agar. Colonies are small, circular, convex and vary between colony morphologies with margins that are entire (smooth colonies) or undulate (rough colonies). Colonies vary in opacity and are 0.5 to 1 mm in diameter after 48 h of growth in 5% CO₂ at 37 °C on GCB agar. The DUS, GCCGTCTGAA, enriched in the type strain's genome confirms membership in the genus.

Type strain is AP2031^T (=DSM 101846^T=CCUG 68283^T=LMG 29261^T) and was isolated from an oral swab of *Mus musculus* subsp. *domesticus* in Tucson AZ, USA. The type strain has a DNA G+C content of 53.3 mol%.

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