Streptobacillus notomytis sp. nov., isolated from a spinifex hopping mouse (Notomys alexis Thomas, 1922), and emended description of Streptobacillus Levaditi et al. 1925, Eisenberg et al. 2015 emend.

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A pleomorphic, Gram-negative, rod-shaped, indole-, oxidase- and catalase-negative, non-spore-forming, non-motile bacterium was isolated in 1979 from the heart of a spinifex hopping mouse (Notomys alexis Thomas, 1922) with septicaemia and stored as Streptobacillus moniliformis in the strain collection of the Animal Health Laboratory, South Perth, Western Australia (AHL 370-1), as well as under CCUG 12425. On the basis of 16S rRNA gene sequence analyses, the strain was assigned to the genus Streptobacillus, with 99.4 % sequence similarity to the type strain of Streptobacillus moniliformis, 95.6 % sequence similarity to the type strain of Streptobacillus hongkongensis and 99.0 % sequence similarity to the type strain of Streptobacillus felis. The clear differentiation of strain AHL 370-1T from Streptobacillus moniliformis, Streptobacillus hongkongensis and Streptobacillus felis was also supported by rpoB, groEL and recA nucleotide and amino acid sequence analysis. Average nucleotide identity was 87.16 % between strain AHL 370-1T and Streptobacillus moniliformis DSM 12112T. Physiological data confirmed the allocation of strain AHL 370-1T to the family Leptotrichiaceae, considering the very similar profiles of enzyme activities and fatty acids compared to closely related species. Within the genus Streptobacillus, isolate AHL 370-1T could also be separated unambiguously from the type strains of Streptobacillus moniliformis, Streptobacillus hongkongensis and Streptobacillus felis by MALDI-TOF mass spectrometry. Two further strains (KWG2 and KWG24) isolated from asymptomatic black rats in Japan were highly similar to AHL 370-1T. On the basis of these data, we propose the novel species Streptobacillus notomytis sp. nov., with the type strain AHL 370-1T (=CCUG 12425T=DSM 100026T=CCM 8593T=EF 12425T).

Abbreviation: ANI, average nucleotide identity.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, gyrB, groEL and recA gene sequences and the complete genome sequence of strain AHL 370-1T are KR001919, KR001957, KR001938, KR001976 and LJRV00000000 (BioSample SAMN04038436), respectively. Other gene sequences generated within this study are summarized in Table S1.

Three supplementary tables and four supplementary figures are available with the online Supplementary Material.
Rat bite fever and Haverhill fever are disease entities caused by *Streptobacillus moniliformis*, which represented, for almost 90 years, a monotypic species within the genus *Streptobacillus* (Levaditi et al., 1925) (*Streptobacillus, Leptotrichiacaeae, Fusobacteriales*) (Elliott, 2007). The bacterial zoonosis rat bite fever is under-reported worldwide and is transmitted predominantly through rat bites and scratches (Gaastra et al., 2009), whereas Haverhill fever represents a second, foodborne form of *Streptobacillus moniliformis* infection that is transmitted by direct or indirect contact with rat urine (Bleich & Nicklas, 2008; Hayashimoto et al., 2008; Torres et al., 2003). Acute symptoms of rat bite fever include fever, malaise, muscle pain, arthritis and abscess formation, endocarditis, bacteraemia and maculopapular, petechial or pustular rash as well as vomiting and pharyngitis (Gaastra et al., 2009). Approximately 50–100 % of wild rats usually carry *Streptobacillus moniliformis* asymptomatically in their oro- or nasopharynx and shed the organism with saliva and urine (Ditchfield et al., 1961; Elliott, 2007; Washburn, 1995), but abscess formation has been also described in rats and mice (Rohde et al., 2008; Wullenweber et al., 1990). Other rodent species besides rats and mice, such as gerbils, squirrels and guinea pigs, as well as companion and exotic animals and livestock, are principally susceptible to infection, but mice may develop clinical disease strain-dependently (Boyer et al., 1958; Das, 1986; Ditchfield et al., 1961; Gaastra et al., 2009; Glünder et al., 1982; Gourlay et al., 1982; Mohamed et al., 1969; Russell & Straube, 1979; Smallwood, 1929; Valverde et al., 2002; Wullenweber et al., 1990; Yamamoto & Clark, 1966).

In the last few years, *Streptobacillus*-like organisms have been noticed beside *Streptobacillus moniliformis*, from which *Streptobacillus hongkongensis* (Woo et al., 2014) and *Streptobacillus felis* (Eisenberg et al., 2014, 2015a) were recently described as novel species causing quinsy and septic arthritis in humans and pneumonia in a cat, respectively. Furthermore, two additional members of the genus *Streptobacillus* were reported, one of which was found in a canine oral microbiome project (sequence COT-370; Dewhirst et al., 2012). The other (OGS16) was isolated from the oral cavity of a Japanese black rat (*Rattus rattus*) (Kimura et al., 2008). We have found evidence for a further novel species, and the respective strain is object of the present description.

Strain AHL 370-1<T> was isolated in 1979 from the heart of a spinifex hopping mouse (*Notomys alexis* Thomas, 1922) with septicaemia (Hopkinson & Lloyd, 1981). AHL 370-1<T> grows after 2–5 days of incubation at 37 °C under a capnophilic atmosphere of 10 % CO₂ on Columbia agar with 5 % sheep blood (SBA; Oxoid). On this agar, strain AHL 370-1<T> was also able to grow weakly at 43 °C, but not at 10, 20 or 50 °C. The strain could also be cultivated on tryptone soy agar (TSA; Oxoid) supplemented with 20 % horse serum and Schaedler agar as well as in liquid media [trypotone soy broth (TSB), brain heart infusion and peptone broth, supplemented with 20 % cattle or horse serum] but not on Gassner or MacConkey agar (all Oxoid). Growth was very fastidious, and colonies are butyrous, dry and heterogeneous, resembling mixed cultures on first impression. Gram staining was done according to the Hucker method, as described previously (Gerhardt et al., 1994). Cell morphological features were observed under a Leitz Diaplan light microscope at ×1000, with cells grown for 3 days at 37 °C on SBA. Gram staining revealed irregular, Gram-negative, pleomorphic, fusiform to filamentous, non-spore-forming, non-encapsulated, non-acid-fast rods that were arranged in chains and clumps, sometimes displaying irregular, lateral bulbar swellings. Single rod-shaped cells were approximately 0.45 ± 0.1 μm wide and 0.83 ± 0.08 μm long.

Two strains (KWG2 and KWG24) isolated from oral swabs of asymptomatic black rats in Japan were highly similar to AHL 370-1<T> and were therefore included in the present study. For phylogenetic analysis, genomic DNA was extracted from a bacterial culture with a commercial kit according to the manufacturer’s instructions (MasterPure Complete DNA and RNA Purification kit; Epicentre) and subjected to whole-genome sequencing.

*De novo* assembly was performed with CLC Genomics Workbench, version 7.5 (CLC Bio). For automatic annotation, we used the RAST server (Aziz et al., 2008). Phylogenetic analysis was performed in ARB release 5.2 (Ludwig et al., 2004) using the 16S rRNA gene-based ‘All-Species Living Tree’ Project (LTP) database (Yarza et al., 2008) release 108 (July 2012). All sequences not included in the LTP database were aligned with the SINA online alignment tool version 1.2.11 (Pruesse et al., 2012) and implemented in the LTP database. Pairwise sequence similarities were calculated in ARB using the ARB neighbour-joining tool without the use of an evolutionary substitution model. Phylogenetic trees were reconstructed with the maximum-likelihood method using RAxML version 7.04 (Stamatakis, 2006) with GTR-Gamma and rapid bootstrap analysis and the maximum-parsimony method using DNA- PARS version 3.6 (Felsenstein, 2005). Both trees were based on 100 replications (bootstrap analysis) (Felsenstein, 2005) and 16S rRNA gene sequence alignments of 1119 nt.

The sequenced 16S rRNA gene fragment of strain AHL 370-1<T> represents a stretch of 1481 unambiguously nucleotides between sequence positions 8 and 1538 (*E. coli* numbering; Brosius et al., 1978).

Strain AHL 370-1<T> shared the highest 16S rRNA gene sequence identity with the type strains of *Streptobacillus moniliformis* (99.4 %), *Streptobacillus hongkongensis* (95.6 %) and *Streptobacillus felis* (99.0 %), followed by *Sneathia sanguinegens* (92.9 %). Sequence similarities to all other taxa were below 91 %. Independent of the treeing method, strain AHL 370-1<T> (and also KWG2 and KWG24) formed a distinct cluster (>90 % bootstrap support) with the type strains of *Streptobacillus moniliformis*, *Streptobacillus hongkongensis* and *Streptobacillus felis* (Fig. 1), clearly separated from the genera *Sneathia*, *Sebaldella* and *Leptotrichia*. Strain AHL 370-1<T> clustered closest to the type strain of *Streptobacillus moniliformis*, which is supported by a high
beside the type strain of *Streptobacillus moniliformis* isolated from different sources (Table S1) were analysed in parallel. All shared identical 16S rRNA gene sequences and did not affect the distinct clustering of strain AHL 370-1T in all trees. Strain AHL 370-1T clustered together with strains KWG2 and KWG24 revealed 98.72 and 96.79 % ANI, thus pointing towards conspecificity. Unique features of strain AHL 370-1T in comparison with other strains of *Streptobacillus moniliformis* were also found by DNA–DNA hybridization and electrophoretic protein patterns during previous studies (Costas & Owen, 1987; Hofmann, 1994). From the results of sequence analysis of the 16S rRNA, gyrB, groEL and recA genes and bootstrap value (97 %). Beside the type strain of *Streptobacillus moniliformis*, six further strains of *Streptobacillus moniliformis* isolated from different sources (Table S1) were analysed in parallel. All shared identical 16S rRNA gene sequences and did not affect the distinct clustering of strain AHL 370-1T in the phylogenetic tree.

Amplification of the specific 16S rRNA gene sequences for strains AHL 370-1T, KWG2 and KWG24 resulted in characteristic amplicon sizes of approximately 269 and 1222 bp, employing the published protocols for *Streptobacillus moniliformis*-specific PCR assays according to Kimura et al. (2008) (primers S5, 5′-CATACTCGGAATAAG-ATGG-3′, and AS2, 5′-GCTTAGCTCCTCTTGTGAC-3′) and Nicklas (primers SbmF, 5′-GAGAGAGCTTTGCAGTTTCAC-3′) and Nicklas (primers SbmR, 5′-GTAACCTCAGGTGCAACT-3′) (cited in Rohde et al., 2008), respectively.

For further clarification of the phylogenetic relationship of strain AHL 370-1T to other species of *Streptobacillus*, phylogenetic analyses based on partial nucleotide and amino acid sequences (Glaeser & Kämpfer, 2015) of the gyrB, groEL and recA genes were performed according to the analysis performed by Woo et al. (2014). Respective nucleotide sequences were aligned according to amino acid sequences using CLUSTAL W (Thompson et al., 1994) implemented in MEGA 5 (Tamura et al., 2011). The correct ORF was obtained by using the full-length gene sequence of *Streptobacillus moniliformis* DSM 12112T as a reference. Pairwise sequence similarities were calculated based on p-distances (calculated without an evolutionary model).

Phylogenetic trees were generated using the maximum-likelihood method with a discrete gamma distribution (+G) with five rate categories and by assuming that a certain fraction of sites are evolutionarily invariable (+I) (for nucleotide sequences) and the Jones–Thornton–Taylor model (JTT; Jones et al., 1992) + G + I (for amino acid sequences). Both trees were based on 100 replications.

Phylogenetic trees based on partial nucleotide and more conserved amino acid sequences (Glaeser & Kämpfer, 2015) of gyrB, groEL and recA showed the formation of monophyletic clusters including all species of *Streptobacillus* in all trees. Strain AHL 370-1T clustered together with strains KWG2 and KWG24 (with high bootstrap support) closest to but in a distinct branch from strains of *Streptobacillus moniliformis* (Figs S1–S3). In addition, nucleotide and amino acid sequence similarities were always considerably lower between strain AHL 370-1T (and KWG2 and KWG24) and strains of *Streptobacillus moniliformis*, *Streptobacillus hongkongensis* and *Streptobacillus felis* (Table S2), thereby clearly indicating the genetic distinctiveness of strain AHL 370-1T.

Instead of weak DNA–DNA hybridization results for members of this genus (Eisenberg et al., 2015b), average nucleotide identity (ANI) was determined according to the method described by Goris et al. (2007). The overall ANI between strain AHL 370-1T and *Streptobacillus moniliformis* DSM 12112T was 87.16 %, and therefore it is evident that they represent separate species (Richter & Rosselló-Móra, 2009). Accordingly, comparisons between strain AHL 370-1T and strains KWG2 and KWG24 revealed 98.72 and 96.79 % ANI, thus pointing towards conspecificity. Unique features of strain AHL 370-1T in comparison with other strains of *Streptobacillus moniliformis* were also found by DNA–DNA hybridization and electrophoretic protein patterns during previous studies (Costas & Owen, 1987; Hofmann, 1994). From the results of sequence analysis of the 16S rRNA, gyrB, groEL and recA genes and
ANI, it is evident that strain AHL 370-1\textsuperscript{T} is different from the genera *Sneathia*, *Sebaldella* and *Leptotrichia* and from the species *Streptobacillus moniliformis*, *Streptobacillus hongkongensis* and *Streptobacillus felis*.

Results from the physiological characterization are given in the species description and in Table 1. Extended biochemical profiling was carried out according to the manufacturers’ instructions using the following commercial test systems: Micronaut Strept\textsuperscript{2} (Merlin Diagnostika; Manafi et al., 1991) and VITEK2-compact with the NHI card and API ZYM (both from bioMérieux). Vitek NHI identified strain AHL 370-1\textsuperscript{T} as *Neisseria elongata* (bio profile 0233000000) and *Streptobacillus moniliformis* DSM 12112\textsuperscript{T}, *Streptobacillus hongkongensis* DSM 26322\textsuperscript{T} and *Streptobacillus felis* 131000547\textsuperscript{T} as *Neisseria cinerea* with 98 % (0232000000), 93 % (0220000040) and 99 % (0220000000) confidence, respectively. Strain AHL 370-1\textsuperscript{T} cannot be differentiated from *S. moniliformis* DSM 12112\textsuperscript{T}, *S. hongkongensis* DSM 26322\textsuperscript{T} and *S. felis* 131000547\textsuperscript{T} by physiological characteristics alone. The antimicrobial susceptibility pattern was determined using MICs obtained by broth microdilution test (Merlin Diagnostika). Results were interpreted according to CLSI MIC criteria based on CLSI MIC interpretive standards for other non-/*Enterobacteriaceae* and anaerobes (CLSI, 2015) according to Table S3. Strain AHL 370-1\textsuperscript{T} was sensitive towards azithromycin, ciprofloxacin, clindamycin, chloramphenicol, erythromycin, gentamicin, meropenem, nalidixic acid, telithromycin and tetracycline, but resistant to trimethoprim/sulfamethoxazole and intermediately resistant to streptomycin.

For MALDI-TOF MS, strains AHL 370-1\textsuperscript{T}, KWG2 and KWG24, *Streptobacillus moniliformis* DSM 12112\textsuperscript{T} and six reference strains of *Streptobacillus moniliformis*, *Streptobacillus hongkongensis* DSM 26322\textsuperscript{T}, *Streptobacillus felis* 131000547\textsuperscript{T} and *Sebaldella termiditis* NCTC 11300\textsuperscript{T} were incubated for 24 h and subsequently selected from the SBA plates and then transferred to steel targets according to manufacturer’s instructions (BrukerBiotyper; BrukerDaltonics). Strains were prepared using the direct smear method provided by the manufacturer. Analysis was performed on a MALDI-TOF MS Biotyper version 3.3.1.0. The database used (DB 5627; BrukerDaltonics) comprised

### Table 1. Physiological characteristics of strains of *Streptobacillus notomytis* sp. nov. and related strains

<table>
<thead>
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<th>Characteristic</th>
<th>1</th>
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<td>Haemolysis on SBA\textsuperscript{*}</td>
<td>–</td>
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<td>–</td>
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<td>+/-</td>
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<tr>
<td>Neuraminidase\textsuperscript{†}</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+/-</td>
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<tr>
<td>Tripeptidase\textsuperscript{†}</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Proline aminopeptidase\textsuperscript{†}</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Hydroxyproline aminopeptidase\textsuperscript{†}</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Glycyl-tryptophan aminopeptidase\textsuperscript{†}</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Arginine aminopeptidase\textsuperscript{†}</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Pyrase\textsuperscript{†}</td>
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<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+/-</td>
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<td>+</td>
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<tr>
<td>Arginine dihydroxylase\textsuperscript{†}</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
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<td>ND</td>
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<tr>
<td>Phosphatase (unspeicified)\textsuperscript{‡}</td>
<td>–</td>
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<tr>
<td>Phenylalanine arylamidase\textsuperscript{‡}</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
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</tr>
<tr>
<td>Ala-Pro-Pro arylamidase\textsuperscript{‡}</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Alkaline phosphatase\textsuperscript{‡}</td>
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<td>–</td>
<td>–</td>
<td>w</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Esterase (C4)\textsuperscript{§}</td>
<td>+</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>+/-</td>
<td>w</td>
<td>+</td>
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<td>Esterase lipase (C8)\textsuperscript{§}</td>
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<td>–</td>
<td>–</td>
<td>+/-</td>
<td>w</td>
<td>w</td>
<td>+</td>
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<tr>
<td>Leucine arylamidase\textsuperscript{§}</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+/-</td>
<td>–</td>
<td>–</td>
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<tr>
<td>x-Chymotrypsin\textsuperscript{§}</td>
<td>+</td>
<td>w</td>
<td>w</td>
<td>+/-</td>
<td>w</td>
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<tr>
<td>Acid phosphatase\textsuperscript{§}</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>w</td>
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<td>+</td>
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<tr>
<td>Naphthol-AS-BI-phosphohydrolase\textsuperscript{§}</td>
<td>–</td>
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<td>w</td>
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<tr>
<td>x-Glucosidase\textsuperscript{§}</td>
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\textsuperscript{*}Results from classical reactions.

\textsuperscript{†}Results obtained by Micronaut Strept\textsuperscript{2} (fermentation) and an individual reaction panel designed for the identification of species of *Streptobacillus* (all from Merlin Diagnostika).

\textsuperscript{‡}Results from VITEK2-compact with the NHI card (bioMérieux).

\textsuperscript{§}Results from API ZYM (bioMérieux). Scores of 0–5 indicate strength of enzymic intensities [0–2, negative (–); 3, weak (w); 4–5, positive (+)].
only one entry from *Streptobacillus moniliformis* DSM 12112<sup>T</sup>. *Streptobacillus moniliformis* DSM 12112<sup>T</sup> and six reference strains of *Streptobacillus moniliformis* were identified to the species level with scores above 2.2. Strain AHL 370-1<sup>T</sup> could also be identified as *Streptobacillus moniliformis*, yielding scores above 2.0. A dendrogram including selected main spectra peak lists (msp) of members of the family *Leptotrichiaceae* from the Bruker database as well as strains AHL 370-1<sup>T</sup>, KGW2 and KGW24, *Streptobacillus moniliformis* DSM 12112<sup>T</sup> and further reference strains, *Streptobacillus hongkongensis* DSM 26322<sup>T</sup>, *Streptobacillus felis* 131000547<sup>T</sup>, *Sneathia sanguinegens* CCUG 38322<sup>T</sup> and *Sebaldella termitidis* ATCC 33386<sup>T</sup> is depicted in Fig. S4; however, this figure shows a separate position of the spectrum from strain AHL 370-1<sup>T</sup> compared with those from *Streptobacillus hongkongensis* and *Streptobacillus felis* as well as all strains from *Streptobacillus moniliformis*.

Fatty acid analysis was carried out according to Kämpfer & Kroppenstedt (1996) (Table 2). The major fatty acids C<sub>16 : 0</sub>, C<sub>18 : 0</sub>, C<sub>18 : 1</sub> and summed feature 5 (anteiso-C<sub>18 : 0</sub> and/or C<sub>18 : 2</sub>) were used. The major fatty acids C<sub>16 : 0</sub>, C<sub>18 : 0</sub>, C<sub>18 : 1</sub> and summed feature 5 (anteiso-C<sub>18 : 0</sub> and/or C<sub>18 : 2</sub>) were used. Summed feature 5 contains anteiso-C<sub>18 : 0</sub> and/or C<sub>18 : 2</sub>. More recently, genetic analyses (Figs 1 and S1–S3) and the differences both the molecular differences obtained by ANI and phylogenetic analyses (Figs 1 and S1–S3) and the differences between species and strains, A. H. L. 370-1<sup>T</sup> could also be identified as *Streptobacillus moniliformis* from the Bruker database as well as strains AHL 370-1<sup>T</sup>, KGW2 and KGW24, *Streptobacillus moniliformis* DSM 12112<sup>T</sup> and further reference strains, *Streptobacillus hongkongensis* DSM 26322<sup>T</sup>, *Streptobacillus felis* 131000547<sup>T</sup>, *S. sanguinegens* CCUG 38322<sup>T</sup> and *Sebaldella termitidis* ATCC 33386<sup>T</sup> is depicted in Fig. S4; however, this figure shows a separate position of the spectrum from strain AHL 370-1<sup>T</sup> compared with those from *Streptobacillus hongkongensis* and *Streptobacillus felis* as well as all strains from *Streptobacillus moniliformis*.

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### Table 2. Cellular fatty acid patterns of strains of *Streptobacillus notomytis* sp. nov. and type strains of the three species of *Streptobacillus*

<table>
<thead>
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<th>Fatty acid</th>
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<th>4</th>
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<th>6</th>
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<tr>
<td>C&lt;sub&gt;14 : 0&lt;/sub&gt;</td>
<td>–</td>
<td>1.2</td>
<td>1.8</td>
<td>–</td>
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<td>1.4</td>
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<tr>
<td>iso-C&lt;sub&gt;15 : 0&lt;/sub&gt;</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>3.0</td>
</tr>
<tr>
<td>C&lt;sub&gt;16 : 0&lt;/sub&gt;</td>
<td>32.5</td>
<td>23.7</td>
<td>23.0</td>
<td>35.4</td>
<td>26.5</td>
<td>31.8</td>
</tr>
<tr>
<td>C&lt;sub&gt;17 : 0&lt;/sub&gt;</td>
<td>1.5</td>
<td>1.1</td>
<td>1.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Summed feature 5*</td>
<td>11.0</td>
<td>18.4</td>
<td>19.4</td>
<td>10.2</td>
<td>5.6</td>
<td>8.8</td>
</tr>
<tr>
<td>C&lt;sub&gt;18 : 1ω9c&lt;/sub&gt;</td>
<td>24.6</td>
<td>27.6</td>
<td>27.7</td>
<td>20.8</td>
<td>30.2</td>
<td>22.7</td>
</tr>
<tr>
<td>C&lt;sub&gt;18 : 0&lt;/sub&gt;</td>
<td>31.9</td>
<td>20.2</td>
<td>18.8</td>
<td>33.5</td>
<td>34.7</td>
<td>30.8</td>
</tr>
<tr>
<td>C&lt;sub&gt;20 : 4&lt;/sub&gt;ω6,9,12,15c</td>
<td>–</td>
<td>1.3</td>
<td>1.3</td>
<td>–</td>
<td>–</td>
<td>1.4</td>
</tr>
</tbody>
</table>

*Summed features are groups of two or three fatty acids that could not be separated using the MID System. Summed feature 5 contains anteiso-C<sub>18 : 0</sub> and/or C<sub>18 : 2</sub>.

### Description of *Streptobacillus notomytis* sp. nov.

*Streptobacillus notomytis* (no.to.my’tis. N.L. gen. n. *notomytis* of *Notomytis*, referring to the isolation of the type strain from the spinifex hopping mouse, *Notomytys alexis*).

Growth occurs after 2–5 days at 37 °C in a capnophilic atmosphere of 10 % CO<sub>2</sub> on SBA, TSB or TSB with 20 % horse serum, but only weak growth is observed on Schaedler and chocolate agar and no growth on Gassner and MacConkey agar. In an anaerobic environment, reduced growth can be observed. Colonies are tiny, dry, butyrous and slightly

### Emended description of the genus

*Streptobacillus Levaditi et al. 1925, Eisenberg et al. 2015*

The description is emended from that given by Eisenberg et al. (2015a). The following features are added. Rods with rounded or pointed ends or pleomorphic bacilli with cocobacillary, bacillary and filamentous forms. Occur singly or form long, wavy chains. Gram-stain-negative. Non-motile. Non-spore-forming. Most strains are dependent on a capnophilic atmosphere containing 5–10 % CO<sub>2</sub> and grow only weakly anaerobically. Few strains are able to grow aerobically. Capable of growth on blood agar and weak growth on chocolate agar but not on MacConkey agar; require blood, serum or ascitic fluid for growth. Optimum temperature for growth 35–37 °C. Most strains are non-anaerobic, some are υ3-anaerobic. Most strains are positive for esterase (C<sub>4</sub>) and esterase lipase (C<sub>8</sub>). Negative for catalase and cytochrome oxidase, indole production and nitrate reduction. The major fatty acids are C<sub>16 : 0</sub> (palmitic acid), C<sub>18 : 0</sub> (stearic acid) and C<sub>18 : 1ω9c</sub> (oleic acid). DNA G+C content is 24.0–28.9 mol%. The type species is *Streptobacillus moniliformis*.

### Description of *Streptobacillus notomytis* sp. nov.

*Streptobacillus notomytis* (no.to.my’tis. N.L. gen. n. *notomytis* of *Notomytis*, referring to the isolation of the type strain from the spinifex hopping mouse, *Notomytys alexis*).

Growth occurs after 2–5 days at 37 °C in a capnophilic atmosphere of 10 % CO<sub>2</sub> in the presence of blood or serum, its negative reactivity for cytochrome oxidase, catalase, nitrate reduction and indole production, the production of a ‘cotton ball’–like appearance in liquid media, its inducible L-forms in addition to ‘normal’ small butyrous colonies, its Gram-negative, filamentous, rod-shaped phenotype arranged in chains and clumps with irregular bulbar swellings and its broad antimicrobial susceptibilities (Tables 1 and S4) also support the placement of the isolate in the genus *Streptobacillus* and distinguish it from *S. sanguinegens* and *‘Leptotrichia annmionii’* (Woo et al., 2014). Moreover, genotypic and phenotypic differences show that strain A. H. L. 370-1<sup>T</sup> represents a novel species different from *Streptobacillus moniliformis*, *Streptobacillus hongkongensis* and *Streptobacillus felis*. For this reason, we propose the novel species *Streptobacillus notomytis* sp. nov. to accommodate strain A. H. L. 370-1<sup>T</sup>. Our results also necessitate an emended description of the genus *Streptobacillus*.
opaque, 0.1–0.4 mm in diameter. Colonies are non-
haemolytic on SBA. Conversion to L-phase or transitional
phase variant may occur spontaneously rarely during cultiva-
tion. In liquid media (e.g. TSB with 20 % horse serum),
streptobacillary growth can be detected after 2–5 days
as typical 'cotton ball'- or 'breadcrum'-like appearance.
Microscopic morphological features are indicative of
Gram-negative, pleomorphic, fusiform to filamentous,
non-spor-forming, non-encapsulated, non-acid-fast rods,
0.45 ± 0.1 μm wide and 0.83 ± 0.08 μm long, that are
arranged in chains and clumps, also sometimes displaying
irregular, lateral bulbar swellings. Positive for esterase C4,
esterase lipase C8, leucine arylamidase, phenylalanine
arylamidase, Ala-Phe-Pro arylamidase and α-chymotrypsin.
Negative for motility, acid phosphatase, alkaline phospha-
tase, lipase (C14), valine arylamidase, cystine arylamidase,
trypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase,
β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronii-
dase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosi-
cerase, cytochrome oxidase, catalase, nitrate reduction and
indole production.

The type strain, AHL 370-1T (=CCUG 12425T=DSM
100026T=CCM 8593T=EF 12425T), was isolated from
the heart of a spinifex hopping mouse (Notomys alexis
Thomas, 1922) with septicaemia (Hopkinson & Lloyd,
1981) and, for priority reasons, the species epithet notomytis
was chosen despite the occurrence of the species also in
Rattus rattus. The G+C content of the DNA of the
type strain is 28.1 mol% and the genome size is
1.76 Mbp. EF refers to the E. Falsen strain collection, Uni-
versity of Göteborg, Sweden.

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