Streptobacillus felis sp. nov., isolated from a cat with pneumonia, and emended descriptions of the genus Streptobacillus and of Streptobacillus moniliformis

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A pleomorphic, Gram-stain-negative, rod-shaped, indole-, oxidase- and catalase-negative, non-spore-forming, non-motile bacterium (strain 131000547 T) was isolated from the lungs of a cat with pneumonia. On the basis of 16S rRNA gene sequence analyses the strain was assigned to the genus Streptobacillus with 97.6 % sequence similarity to the type strain of Streptobacillus moniliformis and 94.6 % to that of Streptobacillus hongkongensis. The clear differentiation of strain 131000547 T from Streptobacillus moniliformis and Streptobacillus hongkongensis was also supported by gyrB, groEL, and recA nucleotide and amino acid sequence analysis. DNA–DNA hybridization demonstrated <19.9 % (reciprocal 28.7 %) DNA–DNA relatedness between strain 131000547 T and Streptobacillus moniliformis DSM 12112 T. Physiological data confirmed the allocation of strain 131000547 T to the family Leptotrichiaceae. Strain 131000547 T has a unique profile of enzyme activities allowing differentiation from the most closely related species. Within the genus Streptobacillus, isolate 131000547 T could also unambiguously be separated from Streptobacillus moniliformis and Streptobacillus hongkongensis by both matrix-assisted laser desorption ionization time-of-flight mass spectrometry and Fourier transform-infrared spectroscopy. On the basis of these data, a novel species of the genus Streptobacillus, Streptobacillus felis sp. nov., is proposed with the type strain 131000547 T ( = DSM 29248 T = CCUG 66203 T = CCM 8542 T). Emended descriptions of the genus Streptobacillus and of Streptobacillus moniliformis are also given.

The genus Streptobacillus (Levaditi et al., 1925) (Leptotrichiaceae; Fusobacteriales) comprised, for almost 90 years, a monotypic species, Streptobacillus moniliformis (Elliott, 2007; Gaastra et al., 2009), one of the two aetiological organisms of rat bite fever (RBF), which is an under-reported, globally occurring bacterial zoonosis (Gaastra et al., 2009). The infection is predominantly transmitted through rat bites and scratches. A second food-borne form of Streptobacillus moniliformis infection named Haverhill fever is transmitted by direct or indirect contact with rat urine (Bleich & Nicklas, 2008; Hayashimoto et al., 2008; Torres et al., 2003). Acute symptoms of RBF 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
asymptomatically carry Streptobacillus moniliformis in their oropharynx or nasopharynx and shed the organism with saliva and urine (Ditchfield et al., 1961; Elliott, 2007; Washburn, 1995), but abscess formation has also been described in rats and mice (Rohde et al., 2008; Wullenweber et al., 1990). Apart from rats and mice, other rodent species such as gerbils, squirrels, spinifex hopping mice or 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; Gländer 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 recent years Streptobacillus-like organisms have been noted, apart from Streptobacillus moniliformis, from which Streptobacillus hongkongensis (Woo et al., 2014) was recently described as a novel species, which causes quinsy and septic arthritis in humans. Two further Streptobacillus species were reported, one of which was found in a canine oral microbe project (sequence COT-370) (Dewhirst et al., 2012). The other is a Streptobacillus species isolated from a cat with pneumonia (Eisenberg et al., 2014a) and this strain is the subject of the present description.

Strain 131000547T was originally isolated from a cat lung after 2–5 days of incubation at 37 °C under a capnophilic atmosphere of 10 % (v/v) CO2 on Columbia agar with 5 % sheep blood (SBA; Oxoid). On this agar, strain 131000547T was able to grow also at 20–43 °C, but not at 10 or 50 °C. The strain could also be cultivated on TSA (Tryptone-soy agar, Oxoid), supplemented with 20 % horse serum, on Schaedler agar as well as in liquid media [tryptone-soy-bouillon (TSB), brain heart infusion and peptone broth, supplemented with 20 % cattle or horse serum], but not on Gassner or MacConkey agar (Oxoid). Good growth could be observed after 48 h. Gram-staining was carried out according to the Hucker method as described previously (Gerhardt et al., 1994). Cell morphological features were observed under a Leitz light microscope (Leitz Diaplan, Leitz; ×1000), with cells grown for 3 days at 37 °C on SBA. Gram-staining revealed irregular Gram-stain-negative, pleomorphic, fusiform to filamentous, non-spore-forming, non-encapsulated, non-acid-fast rods, which 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 ± 0.8 μm long.

For phylogenetic analysis genomic DNA was extracted using a Maxwell 16 FFPE Plus LEV Purification kit (Promega) according to the manufacturer’s instructions. The 16S rRNA gene was PCR-amplified as described elsewhere (Edwards et al., 1989). The PCR product was purified with a QIAquick PCR purification kit (Qiagen) according to the manufacturer’s instructions and sequenced by GATC Biotech (Konstanz, Germany). Phylogenetic analysis was performed in ARB release 5.2 (Ludwig et al., 2004) using the 16S rRNA-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. The alignment of sequences used for tree reconstruction was controlled manually based on secondary structure information. 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 7.0.4 (Stamatakis, 2006) with GTR-GAMMA and rapid bootstrap analysis, and with the maximum-parsimony method using DNAparse version 3.6 (Felsenstein, 2005). Both trees were based on bootstrap analysis with 100 replications (Felsenstein 1985) and 16S rRNA gene sequences between sequence termini 103 and 1356 [numbering according to the Escherichia coli rRNA gene sequence published by Brosius et al. (1978)].

The sequenced 16S rRNA gene fragment of strain 131000547T represents a continuous stretch of 1415 unambiguous nucleotides between sequence positions 9 and 1444 [E. coli numbering (Brosius et al., 1978)].

Strain 131000547T shared highest 16S rRNA gene sequence similarity with the type strains of Streptobacillus moniliformis (97.6 %) and Streptobacillus hongkongensis (94.5 %), followed by Sneathia sanguinegens (91.8 %). Sequence similarities to all other taxa were below 90 %. Indepen-
dently of the treeing method used, strain 131000547T formed a distinct cluster (>80 % bootstrap support) with the type strains of Streptobacillus moniliformis and Streptoba-
cillus hongkongensis (Fig. 1), which was clearly separated from the genera Sneathia, Sebaldella and Leptotrichia. Strain 131000547T clustered most closely with the type strain of Streptobacillus moniliformis, which is supported by a high bootstrap value (100 %). In addition to the type strain of Streptobacillus moniliformis six further strains of Streptoba-
cillus moniliformis isolated from different original sources (Table S1, available in the online Supplementary Material) were analysed in parallel. All shared identical 16S rRNA gene sequences and did not affect the distinct clustering of strain 131000547T in the phylogenetic tree.

Employment of the published protocols for Streptobacillus moniliformis-specific PCR assays, according to Kimura et al. (2008) and Nicklas (cited in Rohde et al., 2008) (Table S2) showed that amplification of the specific 16S rRNA gene sequences for strain 131000547T resulted in characteristic amplicon sizes of approximately 269 and 1222 bp, respectively.

For further clarification of the phylogenetic relationship of strain 131000547T to other species of the genus Strepto-
bacillus phylogenetic analyses based on partial nucleotide and amino acid sequences of gyrB, groEL and recA genes were performed, according to the analysis described 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 12112 T 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 sides are evolutionary 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.

In all phylogenetic trees based on partial nucleotide and more conserved amino acid sequences of gyrB, groEL and recA the formation of monophyletic clusters was shown, indicating all species of the genus Streptobacillus. Strain 131000547 T clustered (with high bootstrap support) most closely, but in a distinct branch, to strains of Streptobacillus moniliformis (Figs S1–S3). In addition, nucleotide and amino acid sequence similarities were always considerably lower between strain 131000547 T and strains of Streptobacillus moniliformis and Streptobacillus hongkongensis (Table S3), clearly indicating the genetic distinction of strain 131000547 T.

For DNA–DNA hybridization, the method described by Ziemke et al. (1998) was used, except that for nick-translation 2 μg DNA was labelled during 3 h of incubation at 15 °C. The overall DNA–DNA relatedness between strain 131000547 T and Streptobacillus moniliformis DSM 12112 T was ≤19.9 % (reciprocal 28.7 %) as determined by hybridization, and therefore it is evident that they represent separate species.

From the results of sequence analysis of the 16S rRNA, gyrB, groEL and recA genes and DNA–DNA hybridization, it is evident that strain 131000547 T is different from the genera Sneathia, Sebaldella and Leptotrichia and from Streptobacillus moniliformis and Streptobacillus hongkongensis.

Results from the physiological characterization are given in the species description and in Table 1. Extended biochemical profiling was carried out according to the manufacturer’s instructions using commercial test systems, i.e. Micronaut Strept2 especially adapted to the growth characteristics of Streptobacillus moniliformis (Merlin Diagnostika) for fermentation (Manafi et al., 1991), VITEK2-compact with the NHI card, and API ZYM (both bioMérieux). Vitek NHI identified strain 131000547 T, Streptobacillus moniliformis DSM 12112 T and Streptobacillus hongkongensis DSM 26322 T as Neisseria cinerea with 99, 98 and 93 % confidence. Strain 131000547 T could be clearly differentiated from Streptobacillus moniliformis DSM 12112 T and Streptobacillus hongkongensis DSM 26322 T, although some minor discrepancies with the results of Woo et al. (2014) remain (e.g. for leucine arylamidase and naphthol-AS-BI-phosphohydrolase). Nevertheless, API ZYM profiles were run with two different batches, so we believe that the observed differences are related to the natural variability within Streptobacillus moniliformis or to different batches of API ZYM tests, or are caused by enzyme differences between strains HKU33 T.
Table 1. Characteristics of strain 131000547T and strains of Streptobacillus moniliformis and Streptobacillus hongkongensis

Taxa: 1, strain 131000547T; 2, Streptobacillus moniliformis DSM 12112T; 3, results from six reference strains of Streptobacillus moniliformis (ATCC 27747, ATCC 49567, ATCC 49940, NCTC 11194, CIP 55-48 and CIP 81-99); 4, Streptobacillus hongkongensis DSM 26322T. +, Positive; −, negative; +/−, variable.

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<td>Tripeptidase†</td>
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<td>α-Glucosidase§</td>
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*Data obtained using classical reactions.
†Data obtained by Micronaut Strep2 (fermentation) and an individual reaction panel designed for the identification of Streptobacillus species.
‡Data obtained using VITEK2-compact with the NHI card.
§Data obtained using the API ZYM system.

Apart from the possibilities mentioned above it could be speculated that this effect could also be attributed to high levels of p-aminobenzoic acid and thymine/thymidine in the plating medium, which are known to antagonize sulfamethoxazole and trimethoprim, respectively (Difco manual).

For matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), strain 131000547T, Streptobacillus moniliformis DSM 12112T, six reference strains of Streptobacillus moniliformis, Streptobacillus hongkongensis DSM 26322T and Sebaldella termitidis ATCC 33386T were incubated for 24 h and subsequently selected from the SBA plates and then subjected to steel-targets, according to the manufacturer’s instructions (BrukerBiotyper; BrukerDaltonics). Strains were prepared using the manufacturer’s direct smear method. Analysis was performed on a MALDI-TOF MS Biotyper Version V3.3.1.0. The database used (DB 4613; BrukerDaltonics) comprised only one entry from Streptobacillus moniliformis DSM 12112T. Streptobacillus moniliformis DSM 12112T and all the reference strains of Streptobacillus moniliformis were identified to the species level with score levels above 2.2. Strain 131000547T could not be identified correctly, yielding score levels of between 1.3 and 1.5. Following the manual inclusion of respective spectra of strain 131000547T to the database these were most closely related to Streptobacillus moniliformis. A dendrogram including selected main spectra peak lists of the family Leptotrichiaceae from the Bruker database, as well as strain 131000547T, Streptobacillus moniliformis DSM 12112T and further reference strains, Streptobacillus hongkongensis DSM 26322T and Sebaldella termitidis ATCC 33386T is depicted in Fig. S4.

Fourier transform-infrared (FT-IR) spectroscopy was also carried out with strain 131000547T, Streptobacillus moniliformis DSM 12112T, Streptobacillus hongkongensis DSM 26322T and Sebaldella termitidis ATCC 33386T. Bacterial isolates were cultured independently in five to seven replicates at 37 °C for 48 h on SBA. Harvesting of cells, preparation of bacterial films on zinc selenide plates, drying and handling were performed as described previously (Kuhm et al., 2009). The dried bacterial films were used directly for examination by FT-IR spectroscopy. IR spectra were recorded for each sample in the transmission mode from 500 to 4000 cm⁻¹ with an FT-IR spectrometer (Tensor27 with HTS-XT-module; BrukerOptics). Acquisition and first analysis of data were carried out using OPUS software (version 4.2; BrukerOptics). The IR spectra of tested strains were compared by cluster analysis [cf. (Eisenberg et al., 2014b; Helm et al., 1991)]. For cluster analysis, the second derivation of the vector-normalized spectra in the wave number range of 500–1400 cm⁻¹ and 2800–3000 cm⁻¹ were used for calculation with Ward’s algorithm (OPUS 4.2) (Ward, 1963). Comparison of the IR spectra of strain 131000547T with those from Streptobacillus moniliformis DSM 12112T, Streptobacillus hongkongensis DSM 26322T and Sebaldella termitidis ATCC 33386T showed a clear separation in two main branches for the species of the genus Streptobacillus and Sebaldella.
termitidis ATCC 33386\textsuperscript{T}. Inside the Streptobacillus branch all spectra from Streptobacillus moniliformis clustered compactly together, closely adjacent to strain 131000547\textsuperscript{T} and Streptobacillus hongkongensis DSM 26322\textsuperscript{T}. The dendrogram obtained depicts the arrangement of isolates in groups according to their spectral differences (Fig. S5).

Both the molecular differences obtained by DNA–DNA hybridization and phylogenetic analyses (Fig. 1 and Figs S1–S3), and the differences based on MALDI-TOF MS and FT-IR (Figs S4 and S5) support the separate position of strain 131000547\textsuperscript{T} as a distinct species of the genus *Streptobacillus*. The dependence of strain 131000547\textsuperscript{T} to grow well in a capnophilic environment with 10 % CO\textsubscript{2} in the presence of blood or serum, its negative reactivity for cytochrome oxidase, catalase, nitrate and indole, the production of a cotton ball-like appearance in liquid media, its inducible L-forms beside ‘normal’ small butyrous colonies, its Gram-stain-negative, filamentous, rod-shaped phenotype arranged in chains and clumps with irregular bulbar swellings and its broad antimicrobial susceptibilities (Table 1 and Table S4) also support the placement of the isolate in the genus *Streptobacillus* and distinguish it from *Sneathia sanguinengens* and ‘Leptotrichia amnionii’ (Woo et al., 2014). Moreover, genotypic and phenotypic differences strongly support strain 131000547\textsuperscript{T} representing a novel species, which differs from *Streptobacillus moniliformis* and *Streptobacillus hongkongensis*. For these reasons we propose that strain 131000547\textsuperscript{T} represents a novel species of the genus *Streptobacillus*, *Streptobacillus felis* sp. nov.

**Emended description of the genus Streptobacillus Levaditi et al. 1925**

The description is emended from that given by Staley & Whitman (2010) and Woo et al. (2014), with the following features 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\textsubscript{2} and grow only weakly. Contrarily, strains from guinea pigs were reported to depend on anaerobic conditions and one out of two strains of *Streptobacillus hongkongensis* was able to grow even anaerobically. Capable of growing on blood agar and weakly on chocolate agar, but not on MacConkey agar; requires blood, serum or ascitic fluid for growth. Optimum temperature for growth is 35–37 °C. Most strains are non-haemolytic, but a *Streptobacillus moniliformis* strain from a rat with otitis as well as the type strains of *Streptobacillus felis* sp. nov. and *Streptobacillus hongkongensis* grow with \( \gamma \)-haemolysis. Most strains are positive for esterase (C4) and esterase lipase (C8). Negative for catalase and cytochrome oxidase, indole production and nitrate reduction.

The type species is *Streptobacillus moniliformis*. The DNA G+C content is 24–26 mol%.

**Description of Streptobacillus felis sp. nov.**

*Streptobacillus felis* (fe’lis. L. gen. n. felis of a cat).

Good growth occurs after 2–5 days at 37 °C in a capnophilic atmosphere of 10 % CO\textsubscript{2} on SBA and TSA or in TSB with 20% cattle or horse serum, but only weak growth on Schaedler and chocolate agar and no growth on Gassner and MacConkey agar. In an anaerobic environment reduced growth is observed. Colonies are tiny, drop-like, shiny and slightly convex, measuring 0.1–0.4 mm in diameter. Colonies are \( \alpha \)-haemolytic on SBA. Conversion to L-phase or transitional phase variants may occur spontaneously during cultivation. In liquid media (e.g. TSB), with the addition of 20 % serum, growth can be detected after 2–4 days with a typical ‘cotton ball’- or ‘bread crumb’-like appearance. Gram-stain-negative, pleomorphic, fusiform to filamentous, non-sporing, non-encapsulated, non-acid-fast rods, 0.45 ± 0.1 \( \mu \)m (width) and 0.83 ± 0.8 \( \mu \)m (length) arranged in chains and clumps; also sometimes irregular and lateral bulbar swellings are displayed. Positive for acid phosphatase, alkaline phosphatase, esterase (C4) and esterase lipase (C8). Negative for motility, phenylalanine arylamidase, ala-pha-pro-arylamidase, lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, \( \gamma \)-chymotrypsin, naphthol-AS-BI-phosphohydrolase \( \alpha \)-galactosidase, \( \beta \)-galactosidase, \( \alpha \)-glucosidase, \( \beta \)-glucosidase, \( \beta \)-glucuronidase, \( \beta \)-acetyl-glucosaminidase, \( \alpha \)-mannosidase, \( \alpha \)-fucosidase, cytochrome oxidase, catalase, nitrate and indole.

The type strain, 131000547\textsuperscript{T} (=DSM 29248\textsuperscript{T}=CCUG 66203\textsuperscript{T}=CCM 8542\textsuperscript{T}), was isolated, in Germany, from a cat with acute suppurative to fibrinous, focally necrotizing bronchopneumonia with multifocal desquamation of type II pneumocytes and alveolar macrophages.

**Emended description of Streptobacillus moniliformis Levaditi et al. 1925**

The description is emended from that given by Staley & Whitman (2010) and Woo et al. (2014). Rods with rounded or pointed ends. Occur singly or form long, wavy chains. Gram-stain-negative, non-motile, non-spore-forming. Conversion to L-phase or transitional phase variants may occur spontaneously during cultivation. Most strains are dependent on a capnophilic atmosphere containing 5–10 % CO\textsubscript{2} and grow only weakly anaerobically. Contrarily, strains from guinea pigs were repeatedly reported to grow exclusively anaerobically. Capable of growing on blood agar and weakly on chocolate agar, but not on MacConkey agar; requires serum or ascitic fluid for growth. Optimum temperature for growth is 35–37 °C. Most strains are non-haemolytic, but a strain from a rat with otitis grows with \( \gamma \)-haemolysis. Ferments glucose to produce acid, but not gas after 3–5 days. Positive for phenylalanine arylamidase, ala-pha-pro-arylamidase, \( \gamma \)-chymotrypsin and esterase lipase (C8). Negative for naphthol-AS-BI-phosphohydrolase, \( N \)-acetyl-\( \beta \)-glucosaminidase, cystine arylamidase, \( \alpha \)-fucosidase, \( \alpha \)-galactosidase, \( \alpha \)-glucosidase, \( \beta \)-glucosidase, \( \beta \)-glucuronidase, \( \beta \)-acetyl-glucosaminidase, \( \beta \)-glucosidase, \( \beta \)-glucuronidase, \( \beta \)-acetyl-glucosaminidase, \( \beta \)-glucosidase,

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lipase (C14), z-mannosidase, trypsin, valine arylamidase, catalase, cytochrome oxidase, indole production and nitrate reduction. Variable for alkaline phosphatase, acid phosphatase, esterase (C4) and leucine arylamidase. Resistant to trimethoprim/sulfamethoxazole (MIC > 8/152 µg ml⁻¹), but sensitive to azithromycin (≥ 0.0625 µg ml⁻¹), ciprofloxacin (= 1 µg ml⁻¹), clindamycin (= 0.25 µg ml⁻¹), chloramphenicol (= 1 µg ml⁻¹), erythromycin (≤ 0.5 µg ml⁻¹), gentamicin (≤ 0.125 µg ml⁻¹), meropenem (= 0.25 µg ml⁻¹), nalidixic acid (= 2 µg ml⁻¹), streptomycin (≤ 1 µg ml⁻¹), teltromycin (≤ 0.125 µg ml⁻¹) and tetracycline (≤ 0.125 µg ml⁻¹).

The type strain is CCUG 13453T (≥ 9901T = ATCC 14647T = CCUG 2469T = DSM 12112T = NCTC 10651T). The DNA G+C content of the type strain is 26.3 mol% (Nolan et al., 2009).

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References


