Streptobacillus ratti sp. nov., isolated from a black rat (Rattus rattus)

Tobias Eisenberg, 1 Koichi Imaoka, 2 Masanobu Kimura, 2 Stefanie P. Glaeser, 3 Christa Ewers, 4 Torsten Semmler, 5 Jörg Rau, 6 Werner Nicklas, 7 Tsutomu Tanikawa 8 and Peter Kämpfer 3

Correspondence
Tobias Eisenberg
tobias.eisenberg@hlh.hessen.de

1Landesbetrieb Hessisches Landeslab, D-35392 Giessen, Germany
2Department of Veterinary Science, National Institute of Infectious Diseases, Tokyo 162-8640, Japan
3Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany
4Institut für Hygiene und Infektionskrankheiten der Tiere, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany
5Robert Koch-Institut, D-13353 Berlin, Germany
6Chemisches und Veterinäruntersuchungsamt Stuttgart, D-70736 Fellbach, Germany
7Deutsches Krebsforschungszentrum, D-69120 Heidelberg, Germany
8Ikari Corporation, Chiba 260-0844, Japan

An indole-, oxidase- and catalase-negative, non-motile bacterium, strain OGS16 T, was isolated from an oral swab of a feral black rat (Rattus rattus) in 2007 in Japan. It stained Gram-negative and had pleomorphic, rod-shaped, non-spore-forming cells. Based on 16S rRNA gene sequence analyses, strain OGS16 T was assigned to the genus Streptobacillus, with 16S rRNA gene sequence similarities of 99.3, 99.0, 98.6 and 95.5 % to the type strains of Streptobacillus moniliformis, Streptobacillus notomytis, Streptobacillus felis and Streptobacillus hongkongensis, respectively. Strain OGS16 T could also be differentiated clearly from other species of the genus Streptobacillus by rpoB, groEL and recA nucleotide and deduced amino acid sequence analysis. DNA–DNA relatedness as obtained by average nucleotide identity was 89.10 % between strain OGS16 T and Streptobacillus moniliformis DSM 12112 T. Chemotaxonomic and physiological data for strain OGS16 T were congruent with results for other closely related members of the family Leptotrichiaceae, represented by highly similar enzyme profiles and fatty acid patterns. MALDI-TOF MS analysis also proved suitable in discriminating strain OGS16 T unequivocally from all currently described taxa of the genus Streptobacillus. On the basis of these data, we propose the novel species Streptobacillus ratti sp. nov., with the type strain OGS16 T (=JCM 31098 T =DSM 101843 T). The G+C content of the DNA of the type strain is 25.9 mol% and the genome size is 1.50 Mbp.

For almost a century, Streptobacillus moniliformis was a monotypic species within the genus Streptobacillus (Levaditi et al., 1925) (Streptobacillus, Leptotrichiaceae, Fusobacteriales). It causes rat bite fever and Haverhill fever (Elliott, 2007). Fever, malaise, muscle pain, arthritis and abscess formation, endocarditis, bacteraemia and...
maculopapular, petechial or pustular rash, as well as vomiting and pharyngitis, are common symptoms of acute rat bite fever (Gaastra et al., 2009). This bacterial zoonosis is transmitted predominantly through rat bites and scratches (Gaastra et al., 2009). Haverhill fever represents a second, foodborne form of *Streptobacillus moniliformis* infection, which is transmitted by direct or indirect contact with rat urine (Bleich & Nicklas, 2008; Regnath et al., 2015). Approximately 50–100 % of wild rats usually carry *Streptobacillus moniliformis* asymptomatically in their oro- or nasopharynx and shed the organism with saliva and urine (Elliott, 2007; Kimura et al., 2008). Other rodents, as well as companion and exotic animal species and livestock, are principally reported to be susceptible to clinical infection in addition to rats and mice, but mice may develop 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).

Recently, *Streptobacillus hongkongensis* (Woo et al., 2014), *Streptobacillus felis* (Eisenberg et al., 2014, 2015b) and *Streptobacillus notomytis* (Eisenberg et al., 2015a) have been described as novel species, all of which cause considerable human or animal sequelae. Furthermore, various phyotypes of *Streptobacillus* consistent with 16S rRNA gene-based operational taxonomic units have been described from Philippine mustard, fish and microbiomes of squirrels, cotton rats, dogs, ducks, dolphins, sea lions and humans (Bik et al., 2010; Chaves-Moreno et al., 2015; Dewhirst et al., 2012; Hullar et al., 2015; Larcia et al., 2011; Maher et al., 1995; Palmer et al., 1994; Strong et al., 2013; Xenoulis et al., 2008) (Fig. S1, available in the online Supplementary Material). A series of strains of *Streptobacillus* spp., KWG2, KWG24 and OGS16T, isolated from Japanese black rats (*Rattus rattus*) (Kimura et al., 2008), was recently found to represent two different species (Eisenberg et al., 2015c). The former two strains, KWG2 and KWG24, are reference strains of *Streptobacillus notomytis*, which was originally isolated from a spinnifex hopping mouse (Eisenberg et al., 2015a). Strain OGS16T gave evidence for a further novel species, and the strain is the subject of the present description.

Strain OGS16T was originally isolated from the oral cavity of a Japanese black rat (Kimura et al., 2008) in a rearing facility of a pest control company (Ikari Corporation, Chiba, Japan). The ancestors of the rats in the facility were captured in 1989 on Chichijima, one of the Ogasawara Islands (Bonin Islands), located some 1000 km south of Japan. OGS16T grew after 1–2 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 OGS16T was also able to grow weakly at 43 °C, but not at 10, 20 or 50 °C. The strain could best be cultivated on TSA (tryptone soy agar; Oxoid) supplemented with 20 % horse serum, but also 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 (all from Oxoid). Growth was fastidious, and colonies were indistinguishable from those of *Streptobacillus moniliformis*, displaying a butyrous, dry appearance. Gram-staining was done according to the Hucker method, as described previously (Gerhardt et al., 1994), and cell morphologies were assessed with cells grown for 3 days at 37 °C on SBA using a Leitz Diplan light microscope at ×1000 magnification. Gram staining revealed Gram-negative, pleomorphic, fusiform to filamentous, non-spore-forming, non-encapsulated, non-acid-fast rods that sometimes displayed irregular, lateral bulbar swellings and were arranged in chains and clumps. Single rod-shaped cells were approximately 0.45 ± 0.1 μm wide and 0.83 ± 0.08 μm long.

Whole-genome sequencing was carried out with strain OGS16T. Genomic DNA was extracted from a 72 h bacterial culture with a commercial kit according to the manufacturer’s instructions [MasterPure Complete DNA and RNA Purification kit (Epicentre), distributed by Biozym Scientific]. *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).

For the first phylogenetic placement, phylogenetic trees based on nearly full-length 16S rRNA gene sequences were reconstructed with MEGAS5 (Tamura et al., 2011) with the maximum-likelihood (ML) method based on the Tamura–Nei model (Tamura & Nei, 1993) and rapid bootstrap analysis and the maximum-parsimony method using the subtree-pruning-regrafting algorithm (Nei & Kumar, 2000). Both trees were based on 1108 nucleotide positions and 100 replications (bootstrap analysis) (Felsenstein, 1985).

The sequenced 16S rRNA gene fragment of strain OGS16T represents a stretch of 1482 unambiguous nucleotides between sequence positions 28 and 1499 (*Escherichia coli* numbering; Brosius et al., 1978). The 16S rRNA gene sequence identity of strain OGS16T was highest with the type strains of *Streptobacillus moniliformis* (99.3 %), *Streptobacillus notomytis* (99.0 %), *Streptobacillus felis* (98.6 %) and *Streptobacillus hongkongensis* (95.5 %), followed by *Sneathia sanguinegens* (93.0 %). Lower (<92 %) sequence similarities were observed to all other taxa. ML as well as maximum-parsimony treeing revealed a distinct cluster (98 % bootstrap support) of strain OGS16T with the type strains of all other species of the genus *Streptobacillus* (Fig. 1), clearly separated from the genera *Sneathia*, *Sebaldeella* and *Leptotrichia*. The closest similarities were observed between strain OGS16T and seven strains of *Streptobacillus moniliformis* from different sources (Table S2) including the type strain, which was supported by a bootstrap value of 78 %. Two previously designed PCR assays for the detection of *Streptobacillus moniliformis* also resulted in characteristic amplicon sizes of approximately
For a more detailed view of the phylogenetic relationships of strain OGS16\textsuperscript{T} and closely related species of the genus Streptobacillus, the criteria of Woo et al. (2014) were considered. Phylogenetic analyses based on both partial nucleotide and deduced amino acid sequences of the gyrB, groEL and recA genes were performed to take into account non-synonymous substitutions (Glaeser & Kämpfer, 2015). Nucleotide and deduced amino acid sequence alignments were generated using CLUSTAL W (Thompson et al., 1994) implemented in MEAG5 (Tamura et al., 2011). Pairwise sequence similarities were calculated based on p-distances (calculated without an evolutionary model). Phylogenetic trees were generated using the ML method with a discrete Gamma distribution (+G) with five rate categories and by assuming that a certain fraction of sites are evolutionary variable (+I) for nucleotide sequences and the Jones–Thornton–Taylor model (JTT; Jones et al., 1992) +G+I for deduced amino acid sequences. All trees were based on 100 replications (bootstrap analysis).

Based on the partial nucleotide sequences and the more-conserved deduced amino acid sequences of gyrB, groEL and recA, phylogenetic trees showed the formation of monophyletic clusters including all species of Streptobacillus in all but the GyrB (deduced amino acid sequence) tree. Strain OGS16\textsuperscript{T} clustered (with high bootstrap support) closest to, but on a distinct branch between, strains of Streptobacillus moniliformis and Streptobacillus notomytis (Figs S2–S4). In the GyrB deduced amino acid sequence tree, strain OGS16\textsuperscript{T} clustered together with all strains of Streptobacillus moniliformis in one clade. The clear genetic distinction of strain OGS16\textsuperscript{T} was supported by a comparison of nucleotide and deduced amino acid sequence differences, which were always considerably lower between strain OGS16\textsuperscript{T} and strains of the other species of the genus Streptobacillus (Table S2).

DNA–DNA hybridization (DDH) of strains of Streptobacillus has been shown to give weak results (Eisenberg et al.)
2015c). Instead, average nucleotide identity (ANI) was determined according to the method described by Goris et al. (2007). The overall DNA–DNA relatedness between strain OGS16T and Streptobacillus moniliformis DSM 12112T was 89.10% as determined by ANI, and it is therefore evident that they represent separate species (Richter & Rosselló-Mora, 2009). The same assertion was supported by a comparison of genomes with the type strains of the remaining species of the genus Streptobacillus, in that ANI between OGS16T and Streptobacillus notomytis AHL 370-1T, Streptobacillus felis 131000547T and Streptobacillus hongkongensis DSM 26322T was calculated as 89.00, 81.96 and 74.77%, respectively. As a countercheck, and to avoid statistical uncertainty, we also confirmed these results by using the in-silico genome-to-genome comparison tool (GGDC 2.0; http://ggdc.dsmz.de/), which works independently of ANI and was found to yield higher correlations with conventional DDH (Meier-Kolthoff et al., 2013). Strain OGS16T consistently displayed DDH estimate levels of $<40.40$% (bootstrap confidence intervals 20.4–38.0%) to strains of all other species of the genus Streptobacillus using formula 2 [identities/high-scoring pair (HSP) length] (data not shown).

Strain OGS16T displayed three conserved signature indels in the amino acid sequences of an MreB/MrI family protein (MreB/MrI; 2 aa deletion), alanine-tRNA ligase (AlaS; 5 aa insertion) and recA (2 aa insertion) that were recently found to be specific for the Leptotrichiaceae (Gupta & Sethi, 2014) (data not shown).

It is evident from the results of 16S rRNA, gyrB, groEL and recA gene sequence analysis, and also from ANI, that strain OGS16T is different from members of the genera Sneathia, Sebaldella and Leptotrichia and from the species Streptobacillus moniliformis, Streptobacillus hongkongensis, Streptobacillus felis and Streptobacillus notomytis.

Physiological results are considered as weak for the unequivocal differentiation of species of the genus Streptobacillus (Eisenberg et al., 2015c). The physiological characterization of strain OGS16T is given in the species description and in Table 1. Biochemical profiling was carried out according to the manufacturer’s instructions using the commercial test systems VITEK2-compact with the NHI card and API ZYM [scores 0–5 indicate the intensity of enzymic reactions (0–2, −; 3, w; 4–5, +)].

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tbody>
<tr>
<td>Haemolysis on SBA</td>
<td>+</td>
<td>−</td>
<td>v</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Phosphatase (unspecified)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Phenylalanine arylamidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Ala-Phe-Pro arylamidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>+</td>
<td>−</td>
<td>w</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Esterase (C4)</td>
<td>−</td>
<td>−</td>
<td>w</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Esterase lipase (C8)</td>
<td>+</td>
<td>+</td>
<td>w/</td>
<td>+</td>
<td>w</td>
<td>+</td>
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<tr>
<td>Leucine arylamidase</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>w</td>
<td>+</td>
</tr>
<tr>
<td>α-Chymotrypsin</td>
<td>+</td>
<td>+</td>
<td>w/</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>−</td>
<td>−</td>
<td>w</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Naphthol-AS-BI-phosphohydrolase</td>
<td>−</td>
<td>−</td>
<td>w</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

*Results were obtained with: a, classical reactions; b, VITEK-compact with the NHI card; c, API ZYM [scores 0–5 indicate the intensity of enzymic reactions (0–2, −; 3, w; 4–5, +)].

susceptibility testing was limited to the assessment of the MIC as obtained by broth microdilution testing (Merlin Diagnostika, as described previously (Eisenberg et al., 2015c). Strain OGS16T displayed MICs as follows (μg ml$^{-1}$): amoxicillin/clavulanic acid ($<2/1$), colistin ($≥1$), florfenicol ($<1$), trimethoprim/sulfamethoxazole ($<0.25/4.75$), tetracycline ($≤0.125$), cephalothin ($<1$), enrofloxacin ($≥2$), erythromycin ($≥1$), penicillin G ($<0.0625$), tiamulin ($<8$), tilmicosin ($<1$, ceftiofur ($≤0.25$), gentamicin ($≥0.5$), spectinomycin ($≥4$), tularthromycin ($<2$) and ampicillin ($≥0.25$).

For matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), strains incubated for 24 h were selected from SBA plates and subsequently transferred to steel targets using the direct transfer protocol according to the manufacturer’s instructions (MALDI Biotyper; Bruker Daltonics). Analysis was performed on a MALDI-TOF MS Biotyper version 3.3.1.0. The database used (DB 5627; Bruker Daltonics) included only one entry from Streptobacillus moniliformis DSM 12112T. With this database alone, strain OGS16T could not be identified, yielding score levels only between 1.3 and 1.5. Following the manual inclusion of spectra from strain OGS16T as well as the type strains of other species of the genus Streptobacillus to the database, all species of Streptobacillus could be differentiated based on spectral differences. Furthermore, MALDI-TOF mass spectra of strain OGS16T turned out to be most closely related
to those of *Streptobacillus notomytis* and *Streptobacillus moniliformis*. A dendrogram including selected main spectra peak (msp) lists of the family Leptotrichiaceae from the Bruker database as well as manual database entries for strain OGS16<sup>T</sup>, six further reference strains of *Streptobacillus moniliformis*, *Streptobacillus hongkongensis* DSM 26322<sup>T</sup>, *Streptobacillus felis* JCM 3100054<sup>T</sup>, *Streptobacillus notomytis* AHL 370-T<sup>2</sup> and *Sebadella termitidis* ATCC 33386<sup>T</sup> is depicted in Fig. S5; it shows a separate position of spectra from strain OGS16<sup>T</sup> from those from other members of the genus *Streptobacillus*.

Fatty acid composition analysis followed Kämpfer & Kropfenstedt (1996) (Table 2). The major fatty acids C<sub>16 : 0</sub>, C<sub>17 : 0</sub>, C<sub>18 : 1ω9c</sub> and C<sub>18 : 2 </sub>(summed feature 5), as reported by Pins et al. (1996), Rowbotham (1983) and Rygg & Bruun (1992), could also be detected in our study, thereby confirming the affiliation of OGS16<sup>T</sup> to *Streptobacillus*.

The separate position of strain OGS16<sup>T</sup> as a member of a distinct species of *Streptobacillus* is well supported by the molecular differences obtained by ANI, phylogenetic analyses of established housekeeping genes (Figs 1 and S2–S4) and differences based on MALDI-TOF MS (Fig. S5). Its growth characteristics are in full agreement with those of other members of the genus. In particular, this includes the fastidious growth of strain OGS16<sup>T</sup> and its dependence on a capnophilic environment with 10 % CO<sub>2</sub> 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 in addition to ‘normal’ small butyrase colonies, its Gram-negative, filamentous, rod-shaped cell morphology arranged in chains and clumps with irregular bulbar swellings, and its presumed broad antimicrobial susceptibilities (Table 1). These characteristics also justify the placement of strain OGS16<sup>T</sup> in the genus *Streptobacillus* and distinguish it from *Sneathia sanguinegens* and *Sneathia annii* (Woo et al., 2014). In summary, genotypic and phenotypic differences prove that strain OGS16<sup>T</sup> represents a novel species different from *Streptobacillus moniliformis*, *Streptobacillus hongkongensis*, *Streptobacillus felis* and *Streptobacillus notomytis*, for which we propose the name *Streptobacillus ratti* sp. nov.

**Description of *Streptobacillus ratti* sp. nov.**

*Streptobacillus ratti* (rat’ti. L. gen. n. ratti of the rat).

Growth can be observed after 1–2 days at 37 °C and depends on a capnophilic atmosphere of 5–10 % CO<sub>2</sub> on SBA or TSA or in TSB with 20 % horse serum, but only weak growth is observed on Schaedler and chocolate agar, and no growth is observed on Gassner or MacConkey agar. In an anaerobic environment, reduced growth can be observed. Colonies are tiny, dry, butyrase and slightly opaque, 0.1–0.4 mm in diameter. Colonies are initially non-haemolytic on SBA but, in aged cultures (> 10 days), a weak zone of α-haemolysis can be observed. Conversion to L-phase or transitional phase variant may rarely occur spontaneously during cultivation. In liquid media, streptobacillary growth can be detected after 2–5 days as a typical ‘cotton ball’- or ‘bread crumb’-like appearance. Microscopic morphological features are indicative of Gram-negative, pleomorphic, fusiform to filamentous, non-spore-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 bulb swellings. Positive for esterase lipase (C8) and α-chymotrypsin. Negative for motility, acid and alkaline phosphatase, esterase (C4), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, x-mannosidase, x-fucosidase, cytochrome oxidase, catalase, nitrate reduction and indole production. 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).

The type strain, OGS16<sup>T</sup> (=JCM 31098<sup>T</sup> =DSM 101843<sup>T</sup>), was isolated from the oral cavity of an asymptomatic black rat (*Rattus rattus*). The G+C content of the DNA of the type strain is 25.9 mol% and the genome size is 1.50 Mbp.

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**Table 2.** Cellular fatty acid patterns of *Streptobacillus ratti* sp. nov. OGS16<sup>T</sup> and the type strains of other species of *Streptobacillus*

<table>
<thead>
<tr>
<th>Fatty acid</th>
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<th>3</th>
<th>4</th>
<th>5</th>
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<tbody>
<tr>
<td>C&lt;sub&gt;14 : 0&lt;/sub&gt;</td>
<td>1.5</td>
<td>1.5</td>
<td>–</td>
<td>1.5</td>
<td>1.6</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;15 : 0&lt;/sub&gt;</td>
<td>–</td>
<td>3.9</td>
<td>3.0</td>
<td>2.1</td>
<td>–</td>
</tr>
<tr>
<td>C&lt;sub&gt;16 : 0&lt;/sub&gt;</td>
<td>28.7</td>
<td>27.8</td>
<td>26.5</td>
<td>28.2</td>
<td>29.4</td>
</tr>
<tr>
<td>C&lt;sub&gt;17 : 0&lt;/sub&gt;</td>
<td>1.5</td>
<td>1.5</td>
<td>–</td>
<td>1.5</td>
<td>–</td>
</tr>
<tr>
<td>Summed feature 5&lt;sup&gt;+&lt;/sup&gt;</td>
<td>8.5</td>
<td>13.3</td>
<td>5.6</td>
<td>12.1</td>
<td>13.0</td>
</tr>
<tr>
<td>C&lt;sub&gt;18 : 1ω6c&lt;/sub&gt;</td>
<td>5.9</td>
<td>2.2</td>
<td>–</td>
<td>2.0</td>
<td>–</td>
</tr>
<tr>
<td>C&lt;sub&gt;18 : 1ω9c&lt;/sub&gt;</td>
<td>23.6</td>
<td>25.1</td>
<td>30.2</td>
<td>24.1</td>
<td>26.6</td>
</tr>
<tr>
<td>C&lt;sub&gt;18 : 0&lt;/sub&gt;</td>
<td>26.3</td>
<td>23.5</td>
<td>34.7</td>
<td>21.6</td>
<td>29.4</td>
</tr>
<tr>
<td>C&lt;sub&gt;20 : 4ω6,9,12,15c&lt;/sub&gt;</td>
<td>–</td>
<td>1.1</td>
<td>–</td>
<td>1.1</td>
<td>–</td>
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<sup>+</sup> Summed feature 5 contains anteiso-C<sub>17 : 0</sub> and C<sub>18 : 1ω9c</sub>.

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