Oceanivirga salmonicida gen. nov., sp. nov., a member of the Leptotrichiaceae isolated from Atlantic salmon (Salmo salar)

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A pleomorphic, Gram-negative, rod-shaped, indole-, oxidase- and catalase-negative, non-spore-forming, non-motile bacterium was originally isolated in 1992 from moribund, seawater farmed Atlantic salmon with multifocal tissue necrosis. Strain AVG 2115T displayed considerable similarities with Streptobacillus moniliformis, one of the two etiological agents of rat bite fever, and has been stored as Streptobacillus sp. NCIMB 703044T. On the basis of 16S rRNA gene sequence analyses, this strain displayed >99 % sequence similarities with uncultured bacterial clones from the digestive tracts of marine mammals, followed by Sneathia sanguinegens CCUG 41628T (92.7 %), ‘Sneathia amnii’ Sn35 (92.5 %), Caviibacter abscessus CCUG 39713T (92.2 %), Streptobacillus ratti OGS16T (91.3 %), Streptobacillus notomytis AHL 370-1T (91.2 %), S. moniliformis DSM 12112T (91.0 %), Streptobacillus felis 131000547T (90.9 %) and Streptobacillus hongkongensis DSM 26322T (89.7 %). Sequence similarities to all other taxa were below 89 %. Phylogenetic analysis for strain NCIMB 703044T revealed highly similar results for gyrB, groEL and recA nucleotide and deduced amino acid sequence analyses independent of the employed treeing method. Average nucleotide identities (ANI) for complete genomes ranged from 66.00 % to 72.08 % between strain NCIMB 703044T and the type strains of Sebaldella termidis, Leptotrichia buccalis, Streptobacillus moniliformis, Sneathia sanguinegens and Caviibacter abscessus. Chemotaxonomic and physiological data of strain NCIMB 703044T were in congruence with closely related members of the family Leptotrichiaceae, represented by highly similar enzyme profiles and fatty acid patterns. MALDI-TOF MS analysis was capable to clearly discriminate strain NCIMB 703044T from all currently described taxa of the family Leptotrichiaceae. On the basis of these data we propose the novel taxon Oceanivirga salmonicida gen. nov. sp. nov. with the type strain AVG 2115T (=NCIMB 703044T) (=DSM 101867T). The G+C content is 25.4 %, genome size is 1.77 Mbp.

Abbreviations: MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; ML, maximum-likelihood; ANI, average nucleotide identity; POCP, percentage of conserved proteins; DDH, DNA–DNA hybridization.

The GenBank/EMBL/DDBJ accession number for the complete genome sequence of Oceanivirga salmonicida gen. nov. sp. nov. NCIMB 703044T is BioProject PRJNA305231 (accession no. SAMN04320974). Other gene sequences generated within this study are summarized in Table S1.

Five supplementary figures and two supplementary tables are available with the online Supplementary Material.
In 1992 and 1993, an unknown bacterial disease occurred on an Atlantic salmon seawater farm in Ireland, causing high mortalities (Palmer et al., 1994). The disease exhibited remarkable pathology, with extensive intracellular bacterial growth, particularly in the kidney endothelial cells. Intra- and extracellular viability of bacteria was indicated by specific histological staining. Bacterial isolates, with consistent growth characteristics, were obtained from kidneys, spleens and livers of a total of 19 fish during the 1992 disease occurrence, and from 16 fish during the 1993 occurrence. However, only one isolate was maintained for further studies (strain AVG 2115T, from 1992). The main pathological features of the disease were reproduced in laboratory-held naïve fish, with intraperitoneal injections of strain AVG 2115T. Unfortunately, the origin of the farmed salmon infection could not be determined. Despite close monitoring, the bacterium was not found on the farm in subsequent years and, to the best of our knowledge, the disease has never re-occurred in Ireland or elsewhere.

Strain AVG 2115T was subsequently 16S rRNA gene sequenced (GenBank: X83517.1), and the highest similarity to published 16S rRNA gene sequences, at that time, was to S. moniliformis (Maher et al., 1995). Indeed, some of the growth characteristics of the isolate resembled those of Streptobacillus, such as the flocculent or ‘cotton ball’ growth in broth and the presence of t-forms (max. colony size of 0.1 mm; glistening, entire, ‘fried egg’ colony appearance). However, some striking physiological differences, e.g. optimum growth temperatures of 15–22 °C and preferred halophilic growth conditions, gave some doubt to any affiliation with the well-known rat bite fever agent. These authors came to the conclusion that strain AVG 2115T possibly represents a novel species or genus within a group of closely related species that now form the family Leptotrichiaceae (Maher et al., 1995). Strain AVG 2115T was deposited by Roy Palmer, Aquatic Veterinary Group, National University of Ireland, Galway, Ireland as Streptobacillus sp. in the NCIMB Culture Collection, Aberdeen, UK (NCIMB 703044T). However, the current study suggests that, based on remarkable DNA heterogeneity with known Leptotrichiaceae species, strain NCIMB 703044T should be assigned to a novel genus of the Leptotrichiaceae family.

Referring to Palmer et al. (1994), strain AVG 2115T initially grew on brain heart infusion agar/broth with 10% foetal calf serum (BHIA-FCS, BHIB-FCS, Difco Laboratories and Oxoid Ltd, UK) and 7% horse blood agar (HBA, Difco Laboratories and Oxoid Ltd), both supplemented with 1% sodium chloride (NaCl). Initial isolation succeeded aerobically at 22 °C after a 4- to 14-day incubation period, with a maximum colony size of 0.3 mm. Anaerobic incubation on these media generally produced a larger maximum colony size of 0.6 mm (Palmer et al., 1994).

In the present study, good growth of strain NCIMB 703044T was observed on tryptone soy agar (TSA, Oxoid, Wesel, Germany) supplemented with 20% horse serum without additional supplementation of NaCl, and weak growth on Columbia agar with 5% sheep blood (SBA; Oxoid) at 15, 20 and 37 °C under a microaerophilic atmosphere, but not at 43 or 50 °C. A very faint incomplete (α)-haemolysis could be observed on HBA and SBA, that turned into complete (β) haemolysis after seven to 14 days of incubation. Strain NCIMB 703044T could also be cultivated on SBA supplemented with nalidixic acid and colistin as well as in liquid media [tryptone soy broth (TSB), supplemented with 20% cattle or horse serum], but not on Schaedler, Gassner, MacConkey and chocolate blood agar supplemented with vitamin K and haemin (all Oxoid). Growth is fastidious and colonies are tiny, dry, and butyraseous and slightly opaque, measuring 0.1–0.6 mm in diameter; older colonies may adopt a concave ‘molar-tooth’ appearance (Fig. 1). 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 TSA supplemented with 20% horse serum. Staining revealed small Gram-negative coco-bacilli 0.40±0.05

\[ \text{Fig. 1. Oceanivirga salmonicida} \] colonies with ‘molar tooth’ appearance. Growth for 14 d at 22 °C, on supplemented brain heart infusion agar. Scale bar =0.25 mm.

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μm (width) and 0.60±0.05 μm (length) on solid media; pleomorphic, fusiform to filamentous, non-spore forming, non-encapsulated, weakly acid-fast rods with a size of 0.40 ±0.05 μm (width) and <5.00 μm (length) in broth that are arranged in chains and clumps.

Electron microscopy was conducted on spleen and kidney samples from the 1992 disease occurrence. Wax embedded tissues were dewaxed, rehydrated, postfixed in 1% osmium tetroxide and embedded in Spurr’s resin. Sections (470 nm) were stained with lead citrate and uranyl acetate and were viewed with a Hitachi 7000 transmission electron microscope. Tissues showed extracellular and intracellular bacteria, of a size consistent with the light microscopy description of cultured cells. The bacteria had triple-membranated cell walls, which lacked the membranous folds or scale-like protrusions described for *Leptotrichia* spp. ([Erbe et al., 2004, Smith et al., 1994]; Fig. 2). In particular, glomerular endothelial cells contained densely packed bacterial cells, within a membrane-bound cytoplasmic vacuole.

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, distributed by Biozym Scientific, Hessisch Oldendorf, Germany) and subjected to whole genome sequencing. De novo assembly was performed with CLC Genomics Workbench, Version 7.5 (CLC Bio, Aarhus, Denmark). For automatic annotation we used the RAST Server: Rapid Annotations using Subsystems Technology (Aziz et al., 2008). Gene sequences from annotated genomes were imported into MEGA5.2.2 (Tamura et al., 2011) and aligned with relevant reference sequences obtained from the NCBI database (http://www.ncbi.nlm.nih.gov) using the ClustalW alignment tool in MEGA5.2.2 with default parameters. For calculation of phylogenetic trees based on nearly full-length 16S rRNA gene sequences, the maximum likelihood (ML) method based on the Tamura-Nei model (Tamura & Nei, 1993) with a discrete Gamma-distribution (+G) with 5 rate categories and by assuming that a certain fraction of sides are evolutionary invariable (+I) was used. Correspondingly, maximum-parsimony (MP) algorithms implemented in MEGA5.2.2 with Subtree-Pruning-Regrafting (Nei & Kumar, 2000) were also calculated and assessed. Tree node reliability was examined by bootstrap analysis using 100 re-samplings for each tree. The sequenced 16S rRNA gene fragment of strain NCIMB 703044 represents an almost continuous stretch of 1515 unambiguous nucleotides between sequence positions 5 to 1548 [E. coli numbering; (Brosius et al., 1978)]. The phylogenetic trees based on 1572 nucleotide (nt) positions (Fig. 3) and 16S rRNA gene sequences between sequence termini 91 and 1402 [numbering according to the *E. coli* rRNA sequence published by (Brosius et al., 1978)]. For Fig. S1 (available in the online Supplementary Material) phylogenetic trees were calculated in ARB release 5.2 (Ludwig et al., 2004) using the ‘all species living tree project’ [LTPs; (Yarza et al., 2008)] database release (LTPs123, September 2015). For this purpose, 16S rRNA gene sequences not included in the database were obtained from GenBank (http://www.ncbi.nlm.nih.gov/), aligned using the SILVA Incremental Aligner [SINA; v1.2.11; (Pruesse et al., 2012)] and implemented into the LTP database tree. Alignments were checked manually considering information of the secondary structure of the 16S rRNA gene sequences. Phylogenetic trees were calculated with the maximum-likelihood method using RAxML version 7.04 (Stamatakis, 2006) with GTR-GAMMA and rapid bootstrap analysis, the maximum-parsimony method using DNAPARS v 3.6 (Felsenstein, 2005), and the neighbor-joining methods using ARB Neighbor-joining and the Jukes-Cantor correction (Jukes & Cantor, 1969). All phylogenetic trees based on 100 re-samplings [bootstrap analysis; (Felsenstein, 1985)]. Only sequence spanning 16S rRNA gene termini 97 to 1356 according to the *E. coli* rrnB numbering, (Brosius et al., 1978) were included in tree calculations. Shorter sequences were not included but added after tree construction into the phylogenetic trees by using the ARB Parsimony quick add option without changing the overall tree topology.

16S rRNA gene sequence analysis clearly supports the affiliation of strain NCIMB 703044 to the family *Leptotrichiaceae* (Figs 3 and S1). It shares highest similarity with an
Fig. 3. Maximum-likelihood tree showing the phylogenetic position of Oceanivirga salmonica strains with the family Leptotrichiaceae. The tree was generated in MEGA5.2.2 based on the Tamura-Nei model (Tamura & Nei, 1993) with a discrete Gamma-distribution (+G) and by assuming that a certain fraction of sides are evolutionary invariable (+I). The tree is based on 1572 nucleotide positions and 16S rRNA gene sequences between sequence termini 91 and 1402 [numbering according to the E. coli 16S rRNA sequence published by (Brosius et al., 1978)]. GenBank accession numbers are given in parentheses. Numbers at branch nodes refer to bootstrap values >70% (100 replicates). Bar, 0.05 nucleotide substitutions per site. Fusobacterium nucleatum subsp. nucleatum ATCC 25586T (NR074442) was used as outgroup.

‘uncultured bacterium clone WDoral10E04’ (99%; KC260719.1) from the mouth of a wild common bottlenose dolphin (Tursiops truncatus) and a series of unpublished sequences from the digestive tracts of wild and captive common bottlenose dolphins and wild California sea lions (Zalophus californianus) (94–99% similarity) (Bik et al., 2008, 2010, 2016), followed by uncultured genome sequences of ‘Sneathia’ sp. clone 123-f47 from a human vagina (95%; Y738659.1), ‘Leptotrichia’ sp. ES2714_GLU’ from unique source (94%; JN647651.1), ‘Streptobacillus’ sp. clone Xi_SPA10’ from cape ground squirrel semen (94%; HM590423.1), ‘Leptotrichia’ sp. clone 123-b6’ from a human vagina (94%; Y738472), the type strain of S. sanguinegens CCUG 41628T (92.7%), ‘Sneathia amnii’ Sn35 (92.5%), C. abscessus CCUG 39713T (92.2%), S. ratti OGS16T (91.3%), S. notonymis AHL370-1T (91.2%), S. moniliformis DSM 12112T (91.0%), S. felis 131000547T (90.9%), and S. hongkongensis HKU33T (89.7%). Sequence similarities to all other taxa are below 89%. For further clarification of the phylogenetic relationship of strain NCIMB 703044T to other members of the Leptotrichiaceae phylogenetic analyses based on both partial nucleotide and deduced amino acid sequences of gyrB, groEL, and recA genes were performed to regard also non-synonymous substitutions (Glaeser & Kämpfer, 2015). Respective nucleotide and deduced amino acid sequences were aligned using ClustalW (Thompson et al., 1994) implemented in MEGA5.2.2 (Tamura, et al., 2011) as described above. Pairwise sequence similarities were calculated based on p-distances (calculated without an evolutionary model). The Jones-Thornton-Taylor model [JTT; (Jones et al., 1992)] +G +I was employed for deduced amino acid sequences. All trees were based on 100 replications (bootstrap analysis).

Using all of the employed phylogenetic methodologies, strain NCIMB 703044T formed a monophyletic cluster (100% bootstrap support) to the type strains of S. sanguinegens CCUG 41628T and C. abscessus CCUG 39713T and also to ‘Sneathia amnii’ Sn35 (Fig. 3) and is also clearly separated from the genera Streptobacillus, Leptotrichia and Sebaldella. Briefly, phylogenetic trees based on partial nucleotide and more conserved deduced amino acid acid sequences of gyrB, groEL and recA showed in all trees the formation of a monophyletic cluster for strain NCIMB 703044T. This strain clustered (with high bootstrap support) closest but in a distinct branch to the Sneathia (S. sanguinegens CCUG 41628T and ‘Sneathia amnii’ Sn35) and C. abscessus clades and always also clearly separated from all Streptobacillus species (Figs S2–S4). Solely in the recA nucleotide and amino acid tree bootstrap support was low and strain NCIMB 703044T clustered, albeit in a separate clade, more closely with Leptotrichia and Sebaldella than with Sneathia type species. In addition, nucleotide and deduced amino acid sequence similarities were always considerably lower between strain NCIMB 703044T and type strains of the genera Caviibacter, Sneathia, Streptobacillus, Sebaldella and Leptotrichia (Table S2), thereby clearly indicating the genetic distinction of strain NCIMB 703044T.

The evaluation of average nucleotide identity (ANI) analyses has proven to be well-suited for species delineation in Streptobacillus species (Eisenberg et al., 2015a, 2016b, 2016c) and superior to DNA-DNA hybridization [DDH;
(Eisenberg et al., 2015b)]. It was carried out according to the method described by Goris et al. (2007). ANI analysis between strain NCIMB 703044T and C. abscessus CCUG 39713T [BioProject PRJNA305231 (BioSample SAMN04320709)], S. sanguinegens CCUG 41628T [PRJNA305231 (SAMN04320708)], S. moniliformis DSM 12112T (CP001779), L. buccalis DSM 1135T (CP001685) and S. termitidis DSM 33386T (CP001739) was 72.08 %, 71.94 %, 69.22 %, 68.11 % and 66.00 %, respectively and therefore significantly below the proposed cut-off for species boundary of 95–96 % DNA heterogeneity (Richter & Rossello-Mora, 2009). The same was true for a comparison of strain NCIMB 703044T with the genomes of further selected type strains, in that ANI values between NCIMB 703044T and ‘Sneathia amnii’ Sn35 (CP011280), S. felis 131000547T [PRJNA304683 (SAMN04306665)], S. notomytis AHL 370-1T [IJRV00000000 (SAMN04308436)], S. ratti OGS16T [LKKW00000000 (SAMN0499675)], and S. hongkongensis DSM 26322T [PRJNA304683 (SAMN04306666)] were calculated with 71.49 %, 70.17 %, 70.27 %, 69.96 % and 69.91 %, respectively, which is clearly indicative for heterospecificity (Richter & Rossello-Mora, 2009). As a countercheck and to avoid statistical uncertainty, we confirmed these results also by using the in-silico genometo-genome comparison tool (GGDC 2.0; http://ggdc.dsmz.de/) that is independently working from ANI and was found to yield higher correlations with conventional DDH (Meier-Kolthoff et al., 2013). Strain NCIMB 703044T constantly displayed DDH estimate levels of 19.2±2.29 to 26.2±2.42 to all type species of the Leptotrichiaceae genera using formula 2 [identities/high-scoring pair (HSP) length], thereby clearly falling below the cut-off for species boundary of 70 % DNA heterogeneity (Johnson, 1984) (data not shown). However, since ANI was found to be not suitable for genus delimitation (Qin et al., 2014), the percentage of conserved proteins (POCP) was assessed to support the designation of a novel genus. POCP calculations for strain NCIMB 703044T versus C. abscessus CCUG 39713T, S. sanguinegens CCUG 41628T

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Table 1. Physiological characteristics of Oceanivirga salmonicida gen. nov. sp. nov. 

<table>
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<tr>
<th>Characters</th>
<th>1</th>
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<th>8</th>
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<tr>
<td>Growth</td>
<td>Haemolysis on SBA§</td>
<td>(+)</td>
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<td>–</td>
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<tr>
<td>10 °C, 15 °C, 22 °C§</td>
<td>+, +, +*</td>
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<td>+</td>
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<td>–</td>
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<td>w</td>
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<td>+/–</td>
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<td>w/+</td>
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<td>–/w</td>
<td>+</td>
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<td>–</td>
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<td>Indole production§</td>
<td>+/-</td>
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</table>

*Data obtained from Palmer et al. (1994).
†NCIMB 703044T, and the type species strains of S. moniliformis, S. sanguinegens and C. abscessus as obtained by VITEK2-compact with the NIH card.
‡API-ZYM™ (both bioMeriex).
§classical reactions
*score values 0–5 indicate strength of enzymatic intensities (0–2: negative [-], 3: weak [w], 4–5: positive [+]).

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and *S. moniliformis* DSM 12112<sup>T</sup> were 46.25, 41.03 and 32.87, respectively, thereby confirming the proposed intergenera POCP boundary of below 50% for prokaryotic lineages (Qin et al., 2014).

PCR assays, that were originally designed for the detection of *S. moniliformis*, were recently found to be rather genus than species specific (Eisenberg et al., 2015b). Interestingly, one of these assays even yielded an amplicon for strain NCIMB 703044<sup>T</sup> in the PCR according to Rohde et al. (2008), but not in the PCR according to Kimura et al. (2008).

Strain NCIMB 703044<sup>T</sup> displayed three conserved signature indels (CSIs) in amino acid sequences of 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* (data not shown) (Gupta & Sethi, 2014).

From the results of the sequence analysis of the 16S rRNA, *gyrB, groEL* and *recA* genes as well as from ANI and POCP analyses it is evident, that strain NCIMB 703044<sup>T</sup> is different from the genera *Caviibacter*, *Neisseria*, *Streptobacillus*, *Leptotrichia* and *Sebalodella* and therefore should be placed into a novel genus.

Results from the physiological characterization are given in Table 1, which includes some of the biochemical and enzymatic results from the case description study of Palmer et al. (1994). Biochemical profiling for this study was carried out according to the manufacturer’s instructions using commercial test systems, i.e., Vitek2-compact with the NHI card (for manufacturer. Analysis was performed on a MALDI-TOF MS, Biotyper Version V3.3.1.0. The database used (DB 5627, BrukerDaltonics, Bremen, Germany). Strains were prepared using the direct transfer protocol provided by the manufacturer. Analysis was performed on a MALDI-TOF MS Biotyper Version V3.3.1.0. The database used (DB 5627, BrukerDaltonics, Bremen, Germany).

### Table 2. Cellular fatty acid pattern of *Oceanivirga salmonicida* gen. nov. sp. nov. NCIMB 703044<sup>T</sup> and type strains of the five *Streptobacillus* species

| Taxa: 1, *Oceanivirga salmonicida* gen. nov. sp. nov. NCIMB 703044<sup>T</sup>; 2, *S. moniliformis* DSM 12112<sup>T</sup>; 3, *S. hongkongensis* DSM 26322<sup>T</sup>; 4, *S. felis* 131000547<sup>T</sup>; 5, *S. notomitis* AML 470-1<sup>T</sup>; 6, *S. ratti* OG516<sup>T</sup>; Biomass for fatty acid analysis was harvested after 3 days of growth in capnophilic environment with 10% CO<sub>2</sub> on Columbia sheep blood agar at 36°C. |
|---|---|---|---|---|---|---|
| Fatty acid | 1 | 2 | 3 | 4 | 5 | 6 |
| C<sub>14:0</sub> | – | 1.5 | – | 1.5 | 1.6 | 1.5 |
| C<sub>15:0</sub> | – | 3.9 | 3.0 | 2.1 | – | – |
| C<sub>16:0</sub> | 25.8 | 27.8 | 26.5 | 28.2 | 29.4 | 28.7 |
| C<sub>17:0</sub> | – | 1.5 | – | 1.5 | – | 1.5 |
| C<sub>18:1ω6c</sub> | 11.6 | 13.3 | 5.6 | 12.1 | 13.0 | 8.5 |
| C<sub>18:1ω7c</sub> | – | 2.2 | – | 2.0 | 26.6 | 5.9 |
| C<sub>18:2ω9c</sub> | 38.2 | 25.1 | 30.2 | 24.1 | 29.4 | 23.6 |
| C<sub>18:3ω6,9,12,15c</sub> | 13.7 | 23.5 | 34.7 | 21.6 | – | 26.3 |
| C<sub>20:4ω6,9,12,15c</sub> | 10.7 | – | – | – | – | – |

The antimicrobial susceptibility testing (AST) pattern was determined for this study using minimal inhibitory concentrations (MIC) obtained by broth microdilution test (Merlin Diagnostika, Bornheim, Germany). Briefly, strain NCIMB 703044<sup>T</sup> was incubated for 5 days at 15°C in TSB supplemented with 20% horse serum and pipetted into commercial microtiter plates for AST. Plates were incubated for four days under identical conditions and AST results revealed the following MIC values (in µg/ml): amoxicillin/clavulanic acid (≤0.125/0.0625), ampicillin (<0.125), cefovecin (≥0.5), cefotiofur (<0.125), cephalothin (<1), chloramphenicol (≥16), clindamycin (≤0.03125), colistin (≥4), enrofloxacin (≥2), erythromycin (≤1), florfenicol (≥8), gentamicin (≥8), oxacillin (≥2), penicillin G (<0.0625), pradofloxacin (≥1), spectinomycin (≥64), tetracycline (<0.0625), tiamulin (<8), tilimicosin (<1), triclosan/sulfamethoxazole (≥4/76) and tulathromycin (<2). This pattern is clearly deviant from MIC values that are typical for members of *Neisathia* and *Streptobacillus* (data not shown).

For matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), strain NCIMB 703044<sup>T</sup>, *C. abscessus* CCUG 39713<sup>T</sup>, *S. sanguinegens* CCUG 41628<sup>T</sup>, *S. moniliformis* DSM 12112<sup>T</sup>, *S. hongkongensis* DSM 26322<sup>T</sup>, *S. felis* 131000547<sup>T</sup>, *S. notomitis* AML 470-1<sup>T</sup>, *S. ratti* OG516<sup>T</sup> and *Sebalodella termitidis* NCTC 11300<sup>T</sup> were incubated for 3–5 d and subsequently selected from the TSA plates supplemented with 20% horse serum and then transferred to steel targets according to manufacturer’s instructions (Bruker-Biotyper, BrukerDaltonics, Bremen, Germany). Strains were prepared using the direct transfer protocol provided by the manufacturer. Analysis was performed on a MALDI-TOF MS Biotyper Version V3.3.1.0. The database used (DB 5627, BrukerDaltonics, Bremen, Germany).
Bruker Daltonics) comprised only one entry from *S. moriliformis* DSM 12112T. With this database alone, strain NCIMB 703044T could not be identified yielding only score levels between 1.3 and 1.5. Following the manual inclusion of respective spectra from strain NCIMB 703044T as well as other *Streptobacillus* and *Sneathia* type strains to the database all these taxa could be differentiated based on spectral differences. Furthermore, MALDI spectra of strain NCIMB 703044T turned out to be most closely related to *C. abscessus*. A dendrogram including selected main spectra peak lists (msp) of the family *Leptotrichiaceae* from the Bruker database as well as manual entries of strains NCIMB 703044T, *C. abscessus* CCGU 39713T, *S. sanguinegens* CCGU 41628T, *S. hongkongensis* DSM 26322T, *S. fels* 131000547T, *S. notomytis* AHL370-1T, *S. ratti* OGS16T and *Sebadella termiditis* ATCC 33386T is depicted in Fig. S5 and shows, however, a clear differentiation of strain NCIMB 703044T with obvious score levels above 2.2 from the other taxa.

Fatty acid composition analysis was carried out for all reference strains according to Table 2 (Kämpfer 1996). The major fatty acids C16:0, C18:0, C18:1ω9c and C18:2ω6c/1ω9c were in accordance with those found for other members of the same family before (Eisenberg et al., 2016a, 2016b, Pins et al., 1996, Rowbotham, 1983, Rygg & Bruun, 1992) Table 2.

Both, the molecular differences obtained by ANI, *in-silico* DDH, POCP and phylogenetic analyses (Fig. 3 and Suppl. Figs. S1-S4) and the differences based on MALDI-TOF MS (Suppl. Fig. S5) and antimicrobial susceptibility pattern support the separate position of strain NCIMB 703044T as a separate genus of the family *Leptotrichiaceae*. The dependence of strain NCIMB 703044T to grow exclusively in the presence of blood or serum, its preference for salinity and colder temperatures, its fastidious growth, and its negative reactivity for cytochrome oxidase, catalase, nitrate and indole, the production of cotton ball-like appearance in liquid media, its inducible phenotype support the placement of the iso-

The type species is *Oceanivirga salmonicida*.

**Description of Oceanivirga salmonicida gen. nov., sp. nov.**

*Oceanivirga salmonicida* (L. *n. salmo*-nis, salmon; L. suff. -cidus (from L. *v. caedo*, to cut or kill), murderer, killer; N.L. *n. salmonicida*, salmon-killer). Growth occurs after 4–14 days at 15–22 °C in an aerobic, microaerophilic or anaerobic atmosphere on BHHA-FCS, BHB-FCS, HBA (all former supplemented with 1 % NaCl, but growth can be observed in 1–4 % NaCl [w/v]), SBA, TSA or TSB with 20 % horse serum, but no growth is observed on Schaedler, chocolate, Gassner and MacConkey agar. Colonies are tiny, dry, butyrate-negative and slightly opaque, measuring 0.1–0.6 mm in diameter; older colonies may adopt a convex ‘molar-tooth’ appearance. Colonies are α-β-haemolytic on HBA and SBA. An i-form also occurs, with colonies of 0.1 mm; glistening, entire, ‘fried egg’ appearance; the cells from these colonies are rounded. In liquid media (e.g. TSB with 20 % horse serum), growth can be detected after 2–5 days as ‘cotton ball’ appearance. With many passages, it was possible to also grow NCIMB 703044T at 37 °C on solid agars and without addition of NaCl to the medium. Microscopic morphological features are indicative of Gram-negative cocco-bacilli (0.40±0.05 µm width and 0.60±0.05 µm length) on solid media. In broth media, the morphology is pleomorphic with fusiform to filamentous, non-sporing, forming, non-encapsulated, weakly acid-fast, non-motile rods (0.40±0.05 µm width and <5.00 µm length), that are arranged in chains and clumps and with filaments showing occasional terminal swellings. Positive for phenylalanine arylamidase, alanine-phenylalanine-proline arylamidase, arginine arylamidase, prolin arylamidase, lysin arylamidase, leucine arylamidase, tyrosin arylamidase, pyrrolidonyl arylamidase, phenylphosphonat, acid phosphatase, arginine dihydrolase and fermentation of glucose, fructose and maltose. Negative for motility, growth in NaCl (5 % [w/v]), V and X factor dependency, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, α-chymotrypsin, trypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, gelatine hydrolysis, Tween 80 hydrolysis, citrate utilization, Voges Proskauer reaction, urease, ornithine decarboxylase, lysine decarboxylase, γ-glutamyl transferase, pyraninamidase, cytochrome oxidase, catalase, nitrate reduction and fermentation of sucrose, ribose, xylose, mannose, mannitol, lactose and glycogen.

The type strain AVG 2115T (=NCIMB 703044T) (=DSM 101867T) was isolated from multifocal necrosis in Atlantic salmon (*Salmo salar*) in Ireland. The G+C content of the DNA of the type strain is 25.4 %, genome size is 1.77 Mbp.
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References


Oceanivirga salmonicida gen. nov., sp. nov.