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Corynebacterium uberis sp. nov. frequently isolated from bovine mastitis

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ABSTRACT

Several strains belonging to the genus *Corynebacterium*, but not to any described species of the genus were isolated from bovine mastitic milk samples over the past five years in the diagnostic unit of the University of Bern. Six of these strains (18M0132^T, 17M2518, 18M0913, 19M0083, 20M1046 and 20M1090) that were phenotypically similar were further characterized genotypically. Gram-positive coryneform rods were catalase positive, facultative anaerobe and CAMP-test negative. Whole genome sequencing and subsequent phylogenetic analysis revealed their genome size to be 2.53 Mb and their G + C content to be between 65.4 and 65.5 mol%. Digital DNA-DNA hybridisation (dDDH) showed the highest similarity of only less than 20% with *Corynebacterium mastitidis* and *Corynebacterium frankenforstense,* which indicated that the isolates belong to an undescribed *Corynebacterium* species. This was confirmed by studying the average nucleotide identity (ANI) where the accepted species boundary is around 95% and which ranged between 70.3% and 74.9% with the most closely related species *C. mastitidis*. We established MALDI-TOF fingerprints of the species, which allows a clear separation from related species and can be used by other laboratories for diagnostic purposes.

Based on our analyses we conclude that the selected strains belong to a previously undescribed species and propose the name *Corynebacterium uberis* sp. nov. The proposed type strain is 18M0132^T (=DSM 111922^T, = CCOS 1972^T).

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Introduction

The genus *Corynebacterium* belongs to the family *Corynebacteriaceae*, order *Mycobacteriales* and contains 136 species to date (https://lpsn.dsmz.de/genus/corynebacterium, accessed 03.01.2022. *Corynebacteriaceae* are Gram-positive, non-acid fast, non-motile straight to curved rods [1]. Their main respiratory quinones are MK-8(H_2) and MK-9(H_2) and the major fatty acids are $C_{16:0}$ and $C_{18:1}$ ω9c while the peptidoglycan is typically of type A1α [1]. After the genus *Turicella* was merged with *Corynebacterium*, *Corynebacterium* is the only genus of the family *Corynebacteriaceae* [2]. *Corynebacteria* can be found in a wide range of ecological niches including soil (e.g. *Corynebacterium (C.) terpenotabidum*), food (e.g. *C. variabile*) and animals including humans, where a number of them are well-known pathogens. The main human pathogen is *C. diphtherie*, which harbours a temperate bacteriophage encoding diphtheria toxin [3]. Two recently described species, namely *C. oculi* and *C. lowii,* were associated with human eye infections [4]. In ruminants several species of *Corynebacterium* are well-known pathogens, such as *C. pseudotuberculosis* causing pseudotuberculosis and *Corynebacterium renale* causing pyelonephritis [5]. A number of species of corynebacteria have been isolated from bovine milk, however the vast majority were *C. bovis* [6,7]. Even though the role of corynebacteria in bovine mastitis has been discussed controversially, an infection with *C. bovis* has been shown to result in increased somatic cell count and decreased lactose concentration [7]. Even though corynebacteria were mostly isolated from subclinically infected quarters, a recent study revealed that 5.8% of *C. bovis* isolates were associated with clinical mastitis [6]. Another species of the genus *Corynebacterium* is *C. mastitidis*, originally isolated from sheep with subclinical mastitis, however it was not frequently reported to be diagnosed in bovine milk samples [8]. This species is closely related to *C. oculi* and *C. lowii* and able to colonize the ocular surface as a commensal [9], but can also act as opportunistic pathogen in the human eye [10].

Abbreviations: ANI, average nucleotide identity; dDDH, digital DNA-DNA hybridization; MALDI-TOF, matrix-assisted laser desorption/ionization – time of flight; MIC, minimal inhibitory concentration; MS, mass spectrometry; TSA SB, Trypticase Soy Agar with 5% sheep blood.

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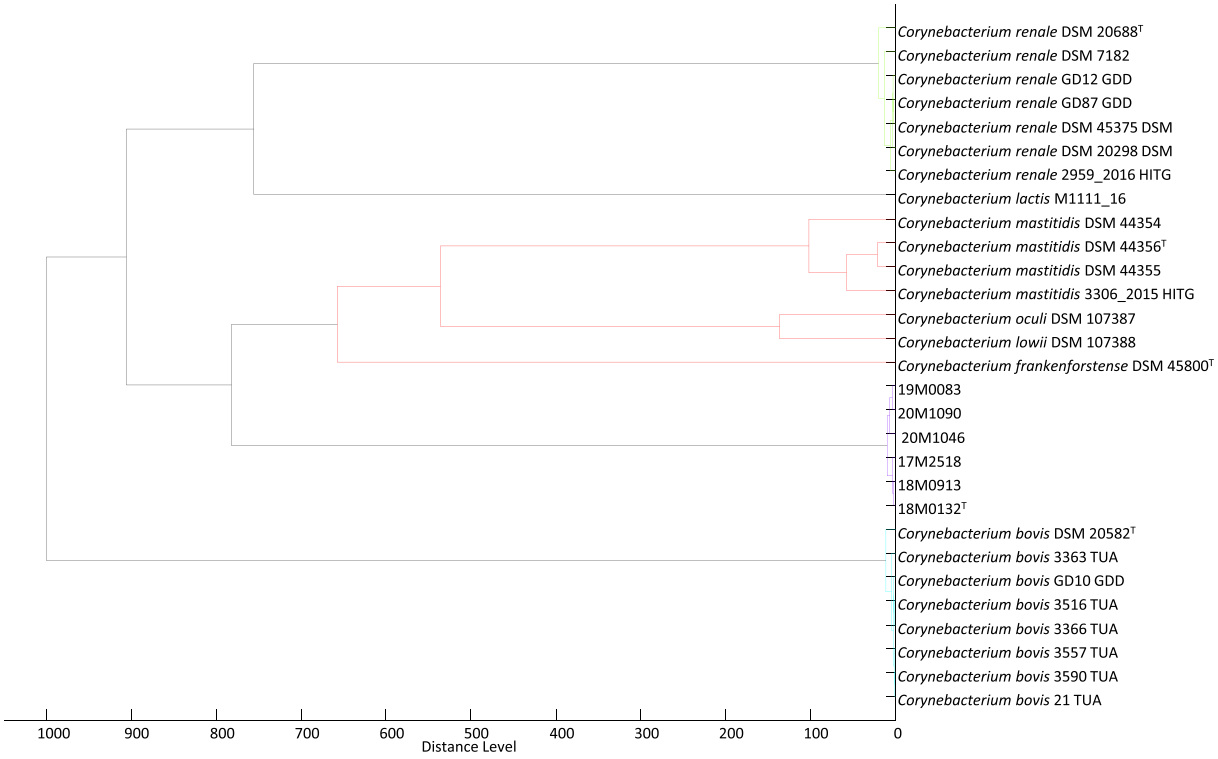


Fig. 1. Dendrogram derived from similarity matrices based on MSP profiles of strains 18M0132^T, 17M2518, 18M0913, 19M0083, 20M1046, 20M1090 and other related species of the genus *Corynebacterium* included in the database. The distance level is normalized to a maximum value of 1000.

Here we describe a novel species of *Corynebacterium* most closely related to the *C. mastitidis*, *C. oculi*, *C. lowii* cluster (Fig. 1). The novel species was isolated frequently (21% of all corynebacteria) from bovine mastitis milk submitted to our laboratory, since we established routine identification via MALDI-TOF MS in 2021. Six representative strains that showed similar biochemical properties were randomly selected to cover different regions and years of isolation and were submitted to extended biochemical characterization and whole genome sequencing. Results of our study confirmed the strains to belong to a single species and to fulfil all criteria to be considered a new species of the genus *Corynebacterium*. Strains 18M0132^T, 17M2518, 18M0913, 19M0083, 20M1046 and 20M1090 are proposed as *Corynebacterium uberis* sp. nov. with type strain 18M0132^T (=DSM 111922^T, = CCOS 1972^T).

Materials and methods

Isolation and ecology

Corynebacterium sp. was isolated from bovine milk specimens submitted to our laboratory for routine diagnosis of agents of clinical or subclinical mastitis. The sampled cattle were kept on farms located in the Swiss cantons of Bern and Fribourg (Table 1). Milk samples (containing 1–10 ml) were centrifuged at 590×*g* for 10 min, supernatants were discarded and one loop full of sediment per specimen was streaked on BD Trypticase Soy Agar with 5% sheep blood (TSA SB, BD) and incubated aerobically (5% CO_2) for 24–48 h at 37 °C. From January to June 2021 all isolates recovered from diagnostic milk specimens were subjected to MALDI-TOF MS to monitor the occurrence of different *Corynebacterium* spp.

MALDI-TOF

MALDI-TOF MS reference spectra (MSP) were generated from fresh cultures (grown aerobically for 72 h on TSA SB at 37 °C) of strains 18M0132^T, 17M2518, 18M0913, 19M0083, 20M1046 and 20M1090 on the microflex® LT (Bruker) following manufacturers' instructions. Additionally, MSPs were produced for *Corynebacterium lactis* M1111_16 (field strain, species verified by 16S rRNA gene sequencing), *C. lowii* DSM 107388^T and *C. oculi* DSM 107387^T. *C. mastitidis*, *C. frankenforstense, C. renale* and *C. bovis* were already present in the library provided by Bruker (8326 MSPs library). Subsequently, a dendrogram based on the MSP similarity matrix was created using MALDI Biotyper Compass Explorer 4.1 (Bruker).

Genomic and phylogenetic characterization

The bacteria were grown for 72 h on TSA SB at 37 °C and DNA was extracted according to the protocol laid out in Pitcher et al. [11] with slight modifications. Briefly, one loop full of colony material was suspended in 500 µl NaCl (0.85%) and pelleted before resuspension in TE buffer (20 mM Tris-HCl; 2 mM EDTA, 12 µl/ml Triton X-100, pH 8). In order to achieve full lysis of the cell wall, 10 µl of 1 mg/ml mutanolysin (Dr. Grogg Chemie, Stettlen-Deisswil, Switzerland) was added in addition to lysozyme (20 mg/ml). Then 250 µl of glass beads (0.5 mm, Sigma-Aldrich) were added followed by vortexing for 5 min and incubated at 37 °C for 1 h before continuing the extraction protocol with addition of GES reagent [11]. All DNA quality control tests, library preparations, size selections and quality assessments, as well as library loading and sequencing steps were performed at the Next Generation Sequencing Platform, University of Bern, Switzerland. Prior to SMRTbell library preparation, bacterial genomic DNA was assessed for quantity, quality and purity using a Qubit 4.0 fluorometer (Qubit dsDNA HS Assay kit; Q32851, Thermo Fisher Scientific), an Advanced Analytical FEMTO Pulse instrument (Genomic DNA 165 kb Kit; FP-1002-0275, Agilent) and a Denovix DS-11 UV–Vis spectrophotometer, respectively. Multiplexed SMRTbell libraries were prepared for sequencing on the Sequel according to the Pac-

Table 1
Genome and isolation data for the six strains described in this study. All strains were isolated as pure culture from milk specimens of bovine mastitis in Switzerland.

Isolate number	Year of Isolation	Canton of isolation	Coverage	Genome size [bp]	Complete and circular	G + C content [mol%]	GenBank accession number
18M0132^T	2018	Bern	120x	2,533,318	yes	65.5	CP085051.1
17M2518	2017	Bern	141x	2,534,678	yes	65.4	CP085050.1
18M0913	2018	Bern	92x	2,527,398	no	65.5	CP085049.1
19M0083	2019	Bern	66x	2,530,639	yes	65.5	CP085048.1
20M1046	2020	Bern	119x	2,532,287	yes	65.5	CP085047.1
20M1090	2020	Fribourg	177x	2,522,849	no	65.5	CP085046.1

Bio guideline and sequencing was carried out on a PacBio Sequel instrument.

Assemblies were preformed using the software Flye 2.7.1 [12] followed by polishing with arrow (GenomicConsensus 2.3.3, https://github.com/PacificBiosciences/GenomicConsensus). For strains where a circular chromosome could be obtained, the sequence was rotated to start with *dnaA* using the fixstart command of circlator 1.5.5 [13].

To verify the authenticity of the genome assembly according to the proposed minimal standards for the use of genome data for the taxonomy of prokaryotes by Chun *et al.* [14] the 16S rRNA gene sequence of 18M0132^T was verified by Sanger sequencing (Microsynth AG, Balgach, Switzerland) following amplification using universal primers [15]. A 1,347 bp sequence was obtained and compared to the genome assembly using BLAST (NCBI) resulting in a 100% identity. The complete 16S rRNA gene sequence was further analyzed using EzBioCloud [16] and NCBI BLAST (database: rRNA_typestrains/16S_ribosomal_RNA). The 50 closest matches from EzBioCloud were downloaded in fasta format and used to create an alignment and a Maximum Likelihood tree using MEGA X 10.1.6 [17].

Assembled genomes were submitted to TYGS type (strain) genome server [18,19] (https://tygs.dsmz.de/, accessed 02.07.2021) for 16S rRNA gene and whole genome based phylogenetic characterization including calculation of digital DNA–DNA hybridisation (dDDH) which has largely replaced the error-prone wet-lab method [18]. For calculation of dDDH formula 6 representing sum of all identities found in high scoring segment pairs divided by total genome length was applied as it best represents the true phylogeny [18].

Furthermore, phylogenetic species tree estimation based on inferred orthologs was performed using OrthoFinder 2.5.2 [20–23]. All assemblies of representative genomes of corynebacteria available in GenBank were downloaded (search terms: "Corynebacterium AND (latest[filter] AND all[filter] NOT anomalous[filter] AND representatives[filter])") and together with the assemblies of the here describes isolates reannotated with Prokka 1.13 [24] prior to running OrthoFinder. Subsequently OrthoFinder was rerun using only strain 18M0132^T as representative to allow comparison of orthogroup statistics between species without over representing the here proposed novel species.

To further verify the phylogenetic position of 18M0132^T as a part of the *C. mastitidis* cluster a Maximum Likelihood phylogeny based on 92 bacterial core genes was obtained using UBCG 3.0 with default options [25].

Average nucleotide identity between the here described isolates and the three most closely related species based on phylogenetic analyses as well as *C. bovis* and *C. renale* was calculated using OAT (OrthoANI Tool version 0.93.1) [26]. To visualize the genome alignment between the six here described strains and the three most closely related species (*C. mastitidis, C. oculi and C. lowii*) BRIG (BLAST Ring Image Generator v0.95) was used.

In order to determine if the six selected strains are clonal a core genome MLST (cgMLST) analysis was performed using chewBBACA 2.5.6 [27]. cgmlst-dists 0.4.0 (https://github.com/tseemann/cgmlst-dists) was subsequently used to obtain the distance matrix. A Neighbour-Joining tree was calculated from the distance matrix using MEGA X 10.1.6 [17].

The genomes of the six strains were further screened for the presence of potential virulence factors using abricate 0.8 (https://github.com/tseemann/abricate) with the virulence factor database (VFDB) setB (DNA sequences, full set 25.03.2022) [28]. The Comprehensive Antibiotic Resistance Database (CARD) was used to screen for resistance genes (https://card.mcmaster.ca/analyze/rgi, accessed 28.05.2022).

Chemotaxonomy

Analyses of whole- cell sugars, respiratory quinones, peptidoglycan structure, mycolic acids and cellular fatty acids including production of the necessary amount of biomass were performed for the proposed type strain (18M0132^T) by the Identification Service at the Leibniz- Institute DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig Germany). The analysis of the peptidoglycan structure was performed according to Schumann et al. [29]. Extraction and high-performance liquid chromatography coupled with diode-array detection and electrospray ionization tandem mass spectrometry (HPLC-DAD-MS) analysis was used for detection of respiratory quinones. Cellular fatty acids were analysed using Sherlock Microbial Identification System (MIS) (MIDI, Microbial ID, Newark, DE 19711 U.S.A.).

Physiology

To determine the biochemical profiles of strains 18M0132^T, 17M2518, 18M0913, 19M0083, 20M1046 and 20M1090 the strains were streaked onto TSA SB agar and incubated for 48–72 h aerobically at 37 °C. Subsequently biochemical profiles were generated using VITEK® 2 ANC card for identification of anaerobic and coryneform bacteria (bioMérieux SA). To allow direct comparison this analysis was also performed for *C. bovis* DSM 20582^T, *C. frankenforstense* DSM 45800^T, *C. mastitidis* DSM 44356^T, *C. lowii* DSM 107388^T, *C. oculi* DSM 107387^T and *C. renale* DSM 20688^T.

Growth of 18M0132^T, 17M2518, 18M0913, 19M0083, 20M1046 and 20M1090 was evaluated aerobically at 18 °C, 25 °C, 30 °C, 37 °C and 44 °C, at 37 °C with 5% CO_2 and anaerobically at 37 °C. Growth was also tested on Brolac agar (Oxoid), in Cation adjusted Mueller Hinton Broth (pH 7.2) (Thermo Scientific), Tryptone Soya Broth (pH 7.3) (Oxoid) and BBL™ Thioglycollate Medium, enriched with vitamin K_1 and hemin (pH 7.0) (BD) at 37 °C. Gram staining was performed using an automated system (Borealys Duo GZ, Axonlab). Catalase activity was tested using the BBL™ Catalase Reagent (BD). CAMP-test was performed with *Staphylococcus aureus* (CCUG 4151). Due to variable results with esculin-hydrolysis in the automated VITEK® 2 system, the esculin test was repeated manually using esculin hydrolysis discs (Rosco Diagnostica A/S, Denmark). Minimal inhibitory concentrations of selected antibiotics were determined by broth microdilution using Sensititre™ test plates

NLD1VMON and NLD3VMON (TREK Diagnostic Systems, Thermo Scientific) and following CLSI guidelines for *Corynebacterium* spp. and coryneforms [30]. Briefly, bacteria were grown for 48 h at 37 °C on TSA SB agar before suspension in cation adjusted Mueller–Hinton broth (Thermo Fisher Scientific) supplemented with 5 % lysed horse blood (Thermo Fisher Scientific) to produce an inoculum of 5×10^5c.f.u. ml^{-1}. The inoculated plates were read after 48 h incubation at 36 ± 1 °C.

Results and discussion

Isolation and ecology

Sediments of diagnostic milk samples were plated onto TSA SB agar plates. After 24–48 h of incubation small white non-haemolytic colonies were visible, very similar to *Corynebacterium bovis*, which is frequently isolated from bovine milk samples. Gram-stain revealed small Gram-positive rods and the catalase test was positive. At this stage, isolates 18M0132^T, 17M2518, 18M0913, 19M0083, 20M1046 and 20M1090 (Table 1) were determined to belong to the genus *Corynebacterium* and were cryoconserved in trypticase soy broth with 30% glycerol at −80 °C in our biobank. After reculturing the strains and subsequent testing for their species level using MALDI-TOF MS (Bruker, MBT 8468 MSP Library), the MS analysis did not provide a species designation so that further analyses were conducted to reveal the taxonomic position of the strains. Subsequently a MALDI-TOF MS reference spectrum (MSP) for 18M0132^T (Supplementary file 1) was generated on the microflex LT (Bruker) to allow identification of additional similar isolates. From 1,380 mastitic milk samples submitted to our laboratory (between January and June 2021), 190 (14%) contained live corynebacteria. Of the latter 69% were *C. bovis*, 21% matched the MSP of isolate 18M0132^T, 2% were *C. amycolatum* and 8% other species. This indicates that the here proposed undescribed species is often associated with mastitic samples, though we did not investigate milk samples from healthy animals, since such samples normally do not get submitted to our diagnostic laboratory. Therefore,

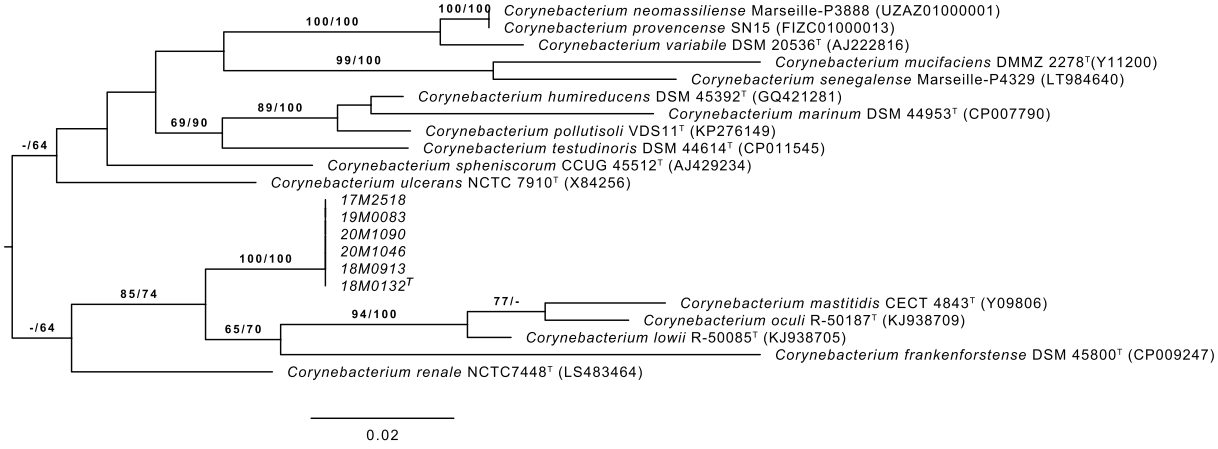
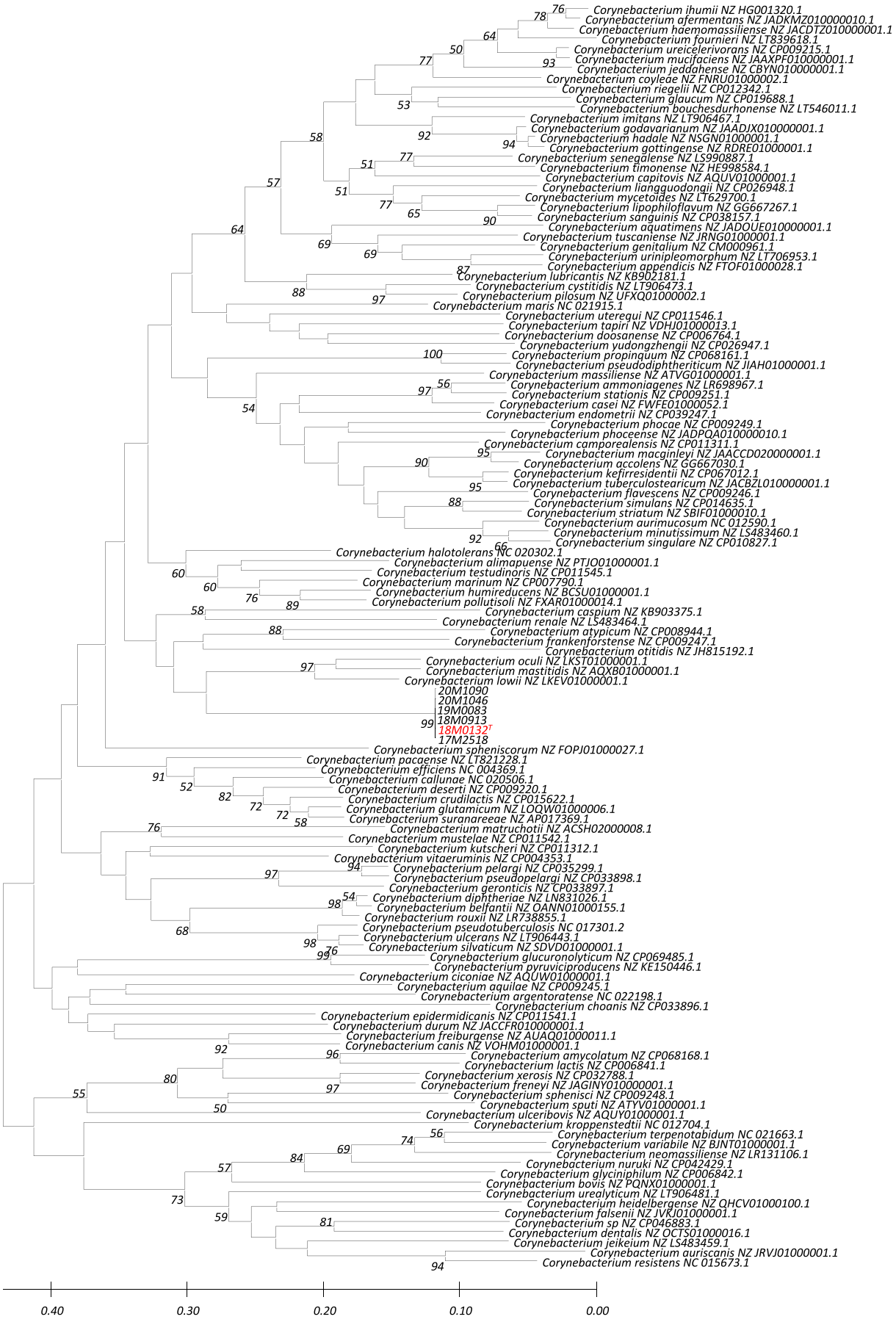


Fig. 2. 16S rRNA gene based phylogeny as provided by TYGS type (strain) genome server [19]. ML (Maximum Likelihood) tree inferred under the GTR+GAMMA model and rooted by midpoint-rooting. The branches are scaled in terms of the expected number of substitutions per site. The numbers above the branches are support values when larger than 60% from ML (left) and MP (maximum parsimony) (right) bootstrapping.

Table 2
Results of Digital DNA –DNA hybridisation (dDDH) as well as average nucleotide identity (ANI). Strain 18M0132^T was compared to the other strains of the potentially new species as well as to phylogenetically related corynebacteria. dDDH is presented as provided by the TYGS type (strain) genome server [18,19] (https://tygs.dsmz.de/, accessed 02.07.2021). For calculation of dDDH formula six representing sum of all identities found in high scoring segment pairs divided by total genome length was applied as it best represents the true phylogeny [18]. ANI was determined by OAT (OrthoANI Tool version 0.93.1) using both the OthoANI and original ANI algorithm.

Subject strain	dDDH (d6, in %)C.I. (d6, in %)	OrthoANI OriginalANI
20M1090	100	99.95%
	[100.0–100.0]	99.93%
19M0083	100	99.96%
	[100.0–100.0]	99.96%
20M1046	100	99.96%
	[100.0–100.0]	99.95%
18M0913	100	99.94%
	[100.0–100.0]	99.94%
17M2518	100	99.95%
	[100.0–100.0]	99.93%
Corynebacterium mastitidis DSM 44356^T (GCA/GCF_000375365)	16.4	74.91%
	[13.8–19.3]	74.15%
Corynebacterium frankenforstense ST18^T (GCA/GCF_001941485)	15.6	74.29%
	[13.1–18.5]	73.87%
Corynebacterium oculi NML 130210^T (GCA/GCF_001412105)	15.1	73.98%
	[12.6–18.0]	73.16%
Corynebacterium lowii LMG 28276^T (GCF_001412085)	14.7	73.42%
	[12.2–17.6]	72.69%
Corynebacterium renale DSM 20688^T (GCA_900478035)	13.7	70.87%
	[11.3–16.5]	70.41%


Corynebacterium ihumii NZ HG001320.1
Corynebacterium afermentans NZ JADKMZ010000010.1
Corynebacterium haemomassiliense NZ JACDTZ010000001.1
Corynebacterium fournieri NZ LT839618.1
Corynebacterium ureicelerivorans NZ CP009215.1
Corynebacterium mucifaciens NZ JAAXPF010000001.1
Corynebacterium jeddahense NZ CBYN010000001.1
Corynebacterium coyleae NZ FNRU01000002.1
Corynebacterium riegelii NZ CP012342.1
Corynebacterium glaucum NZ CP019688.1
Corynebacterium bouchesdurhonense NZ LT546011.1
Corynebacterium imitans NZ LT906467.1
Corynebacterium godavarianum NZ JAADJX010000001.1
Corynebacterium hadale NZ NSGN01000001.1
Corynebacterium gottingense NZ RDRE01000001.1
Corynebacterium senegalense NZ LS990887.1
Corynebacterium timonense NZ HE998584.1
Corynebacterium capitovis NZ AQUV01000001.1
Corynebacterium liangguodongii NZ CP026948.1
Corynebacterium mycetoides NZ LT629700.1
Corynebacterium lipophiloflavum NZ GG667267.1
Corynebacterium sanguinis NZ CP038157.1
Corynebacterium aquatimens NZ JADOUE010000001.1
Corynebacterium tuscaniense NZ JRNG01000001.1
Corynebacterium genitalium NZ CM000961.1
Corynebacterium urinipleomorphum NZ LT706953.1
Corynebacterium appendicis NZ FTOF01000028.1
Corynebacterium lubricantis NZ KB902181.1
Corynebacterium cystitidis NZ LT906473.1
Corynebacterium pilosum NZ UFXQ01000002.1
Corynebacterium maris NC 021915.1
Corynebacterium uteregui NZ CP011546.1
Corynebacterium tapiri NZ VDHJ01000013.1
Corynebacterium doosanense NZ CP006764.1
Corynebacterium yudongzhengii NZ CP026947.1
Corynebacterium propinquum NZ CP068161.1
Corynebacterium pseudodiphtheriticum NZ JIAH01000001.1
Corynebacterium massiliense NZ ATVG01000001.1
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Corynebacterium endometrii NZ CP039247.1
Corynebacterium phocae NZ CP009249.1
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Corynebacterium oculi NZ LKST01000001.1
Corynebacterium mastitidis NZ AQXB01000001.1
Corynebacterium lowii NZ LKEV01000001.1
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20M1046
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18M0913
18M0132T
17M2518
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Corynebacterium efficiens NC 004369.1
Corynebacterium callunae NC 020506.1
Corynebacterium deserti NZ CP009220.1
Corynebacterium crudilactis NZ CP015622.1
Corynebacterium glutamicum NZ LOQW01000006.1
Corynebacterium suranareeae NZ AP017369.1
Corynebacterium matruchotii NZ ACSH02000008.1
Corynebacterium mustelae NZ CP011542.1
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Corynebacterium falsenii NZ JVRJ01000001.1
Corynebacterium sp NZ CP046883.1
Corynebacterium dentalis NZ OCTS01000016.1
Corynebacterium jeikeium NZ LS483459.1
Corynebacterium auriscanis NZ JRVJ01000001.1
Corynebacterium resistens NC 015673.1
0.40
0.30
0.20
0.10
0.00

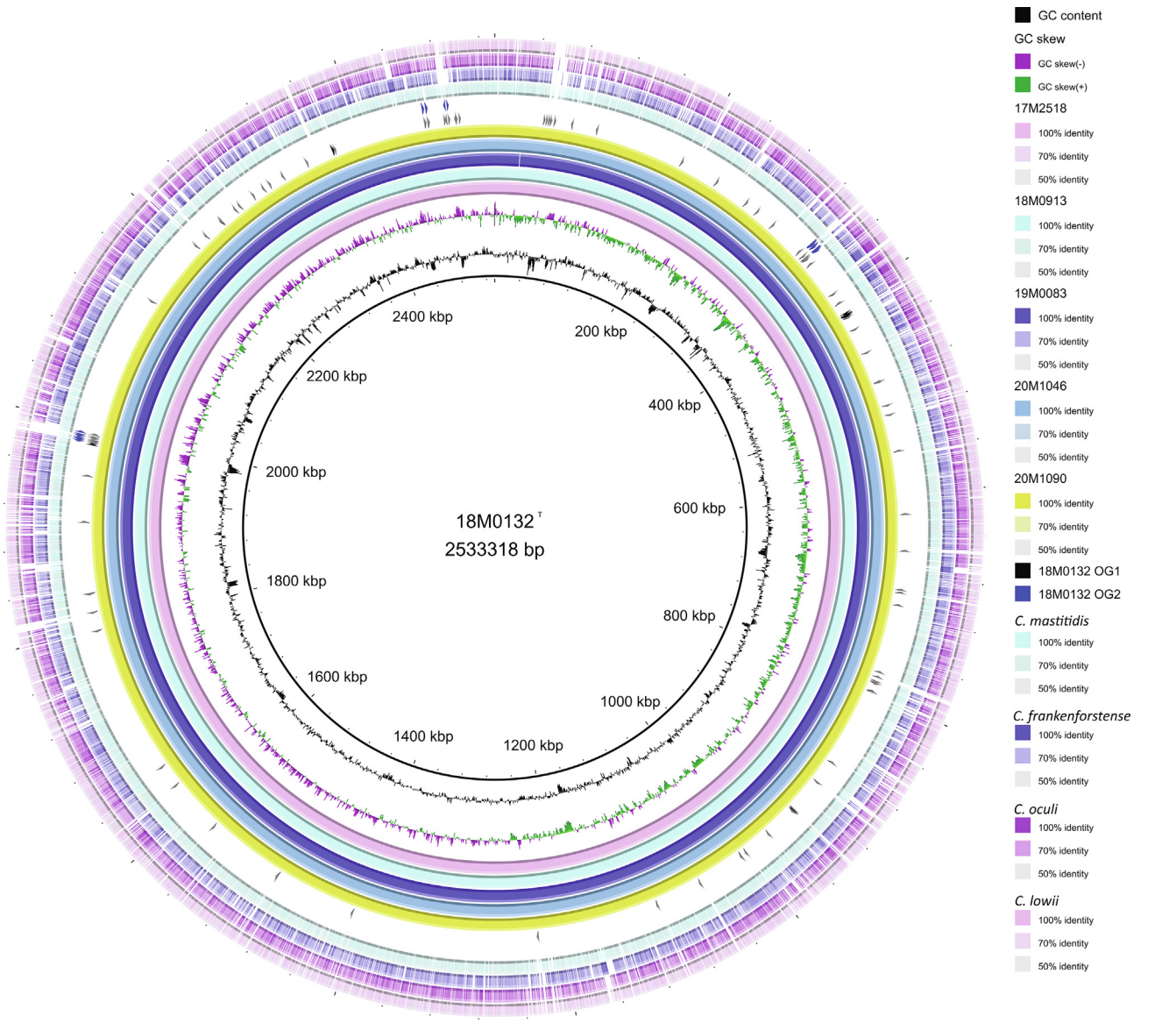

Fig. 4. Circular map of strains 18M0132^T, 17M2518, 18M0913, 19M0083, 20M1046, 20M1090, *C. mastitidis, C. frankenforstense, C. oculi* and *C. lowii* using BLAST Ring Image Generator (BRIG). 18M0132^T was used as the reference. Ring OG1 shows genes belonging to species specific orthogroups as determined by OrthoFinder when including all six strains. Hypothetical proteins are shown in grey, proteins described in Table 3 are shown in black. Ring OG2 shows genes belonging to species specific orthogroups as determined by OrthoFinder when using strain 18M0132^T as representative.

the proposed species is likely to play a role in bovine mastitis. It was not reported before, probably due to the absence of a species description or wrong species designation. However, to unambiguously define its role towards bovine mastitis *in vivo* experiments using the natural host are required.

MALDI-TOF Mass Spectrometry

MSPs were successfully obtained for all strains. The dendrogram based on MSPs (Fig. 1) shows a similar clustering to sequence based phylograms described below. Strains 18M0132^T, 17M2518, 18M0913, 19M0083, 20M1046 and 20M1090 form a clearly separated cluster meaning that these strains belong to a single species, which is likely to be a new one. This so far undescribed species can consistently be identified by MALDI-TOF MS (Bruker), which is the standard method used by many diagnostic laboratories in Switzerland.

Genomic and phylogenetic characterization

Complete circular genomes were obtained for four strains, while two strains could be assembled into a single contig but not circularized due to the read length not spanning the rRNA operon. The genome size of 2.53 Mb was similar in all isolates and they possessed a G+C content of 65.4–65.5 mol% (Table 1).

The most closely related species based on the 16S rRNA gene sequence (NCBI BLAST rRNA_typestrains/16S_ribosomal_RNA, 08.07.2021) were *Corynebacterium mastitidis* S-8^T NR_026376.1

Fig. 3. Phylogenetic tree based on inferred orthologs as determined by OrthoFinder 2.5.2. strains 18M0132^T (marked in red), 17M2518, 18M0913, 19M0083, 20M1046 and 20M1090 are most closely related to the *C. mastitidis - C. oculi - C. lowii* cluster.

(95.96% identity), *Corynebacterium lowii* R-50085^T NR_151864.1 (95.93% identity) and *Corynebacterium renale* Charita-a^T NR_037069.1 (95.84% identity). The 16S rRNA gene also showed a 100% match to *Corynebacterium* sp. (GenBank: KJ938715), which was isolated from a human breast abscess, pointing towards an affinity of the undescribed species to the mammary tissue. An EzBioCloud (21.03.2022) analysis of the 16S rRNA gene sequence revealed the closest match with *Corynebacterium lowii* R-50085^T KJ938705 (96.97% similarity), *Corynebacterium renale* DSM 20688^T PDJF01000001 (96.41% similarity) and *Corynebacterium mastitidis* CECT4843^T Y09806 (96.19% similarity). EzBioCloud similarity values differ from BLAST identity due to EzBioCloud using an algorithm optimized for taxonomic purposes [31].

In the 16S rRNA gene based Maximum Likelihood phylogenetic tree obtained from the TYGS type (strain) genome server (Fig. 2) it can be seen that the *C. mastitidis* - *C. oculi* - *C. lowii* group and *C. frankenforstense* are the closest phylogenetic relatives to the undescribed species forming a cluster together, while *C. renale* (despite high 16S rRNA gene similarity), is located in a related but separate cluster. This clustering was confirmed by the Maximum Liklihood phylogeny based on the 50 closest matches of the EzBioCloud analysis (Supplementary Fig. 1). It is also consistent with results for dDDH where, based on formula 6, the highest match was obtained with *C. mastitidis* DSM 44356^T (16.4%) and *C. frankenforstense* ST18^T (15.6%) (Table 2). This dDDH score is far below the DNA homology of >70% that defines the same species level [18]. ANI with the phylogenetically most closely related species *C. mastitidis, C. lowii* and *C. oculi,* ranged between 72.7% and 74.9% (Table 2) which is also clearly below the generally accepted species boundary of 95% [32] and even the lowest proposed boundary of 93% [33] and confirms the dDDH results. The species tree inferred by OrthoFinder from the 642 orthogroup gene trees that included all species confirmed the phylogenetic position of the here described strains as most closely related to the *C. mastitidis* cluster rather than *C. renale* (Fig. 3). The same clustering was also observed in the core genome-based tree obtained with UBCG (Supplementary Fig. 2).

OrthoFinder assigned 97.2% of genes to 8,229 orthogroups when all six here analysed strains were include with the 122 selected *Corynebacterium* spp., while this number was 8,118 when only the strain 18M0132^T was included since this way potential species specific orthogroups represented by single genes were excluded. The analysed strains contained between 2,252 and 2,255 genes assigned to orthogroups and 2,035 orthogroups were found in all six strains. Ninety-eight genes classified into 90 orthogroups were not present in any of the other species and might thus be considered species specific. This interpretation has to be done with caution, however, as only single strains were included for the other species. The position of the genes in the genome of 18M0132^T and the comparison with strains 17M2518, 18M0913, 19M0083, 20M1046 and 20M1090 as well as their closest relatives *C. mastitidis, C. frankenforstense, C. oculi and C. lowii* is shown in Fig. 4. Ninety-three of the 98 genes were annotated as hypothetical proteins by Prokka (Fig. 4, Table 3).

The genome organisation of the six analysed strains is highly conserved (Supplementary Fig. 3), however, the cgMLST analysis shows that the isolates are not clonal (Fig. 5).

All analyses of genome data clearly show that strains 18M0132^T, 17M2518, 18M0913, 19M0083, 20M1046 and 20M1090 represent one species, which can be classified as a novel species, clearly separated from the phylogenetically closest relatives the *C. mastitidis*-*C. oculi*-*C. lowii* group.

No close matches with known virulence or resistance-encoding genes in the VFDB or CARD respectively were found.

Chemotaxonomy

Chemotaxonomy was performed for strain 18M0132^T. Galactose, arabinose and ribose were detected in the whole-cell sugar preparation, the presence of galactose and arabinose being typical for corynebacteria [1]. HPLC- DAD- MS analysis showed the follow-

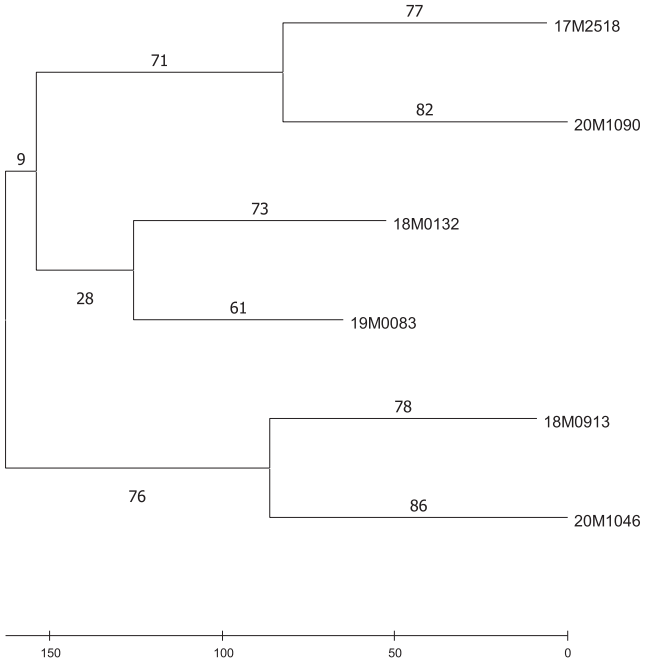

Fig. 5. cgMLST dendrogram. Core genome MLST (cgMLST) analysis was performed using chewBBACA 2.5.6 [27]. cgmlst-dists 0.4.0 (https://github.com/tseemann/cgmlst-dists) was subsequently used to obtain the distance matrix. A Neighbour-Joining tree was calculated from the distance matrix using MEGA X 10.1.6 [17].

Table 3
Non hypothetical proteins (as determined by Prokka) found in 18M0132^T, 17M2518, 18M0913, 19M0083, 20M1046 and 20M1090 belonging to species-specific orthogroups as classified by OrthoFinder.

Position in 18M0132^T (CP085051)	Prokka annotation	Blastp (best match)
411832–412995	Elloramycin glycosyltransferase ElmGT	MULTISPECIES: glycosyltransferase [unclassified *Kocuria*] WP_129360602 (53.95% identity, query cover 97%)
413023–416472	Multidrug efflux ATP-binding/ permease protein	salmochelin/enterobactin export ABC transporter IroC [*Pantoea* sp. Acro-835] WP_167018527.1 (32.42% identity, query cover 97%)
938974–939603	Cell division ATP-binding protein FtsE	ATP-binding cassette domain-containing protein [*Cutibacterium modestum*] WP_221265122.1 (42.56% identity, query cover 93%)
1982274–1982522	Oligoribonuclease	oligoribonuclease [*Corynebacterium* sp. 3HC-13] WP_224400636.1 (67.57% identity, 45% query cover)
2369950–2370714	Biotin transport ATP-binding protein BioM	ATP-binding cassette domain-containing protein [*Yimella* sp. cx-573] MBD2759646.1 (36.74% identity, 79% query cover)

ing menaquinones (MK): MK-7 (0.6%), MK-7(H_2) (4.3%), MK-8 (2.9%), MK-8(H_2) (43.8%), MK-9 (1.9%), MK-9(H_2) (45.5%), MK-10 (H_2) (1.0%) and MK-11(H_2) (<0.1%). MK-9(H_2) and MK-8(H_2) as predominant menaquinones is typical of the genus *Corynebacterium* [1]. No mycolic acids were detected. The peptidoglycan type is A1γ meso-DPM – direct (A31 according to www.peptidoglycan-types.info). This is consistent with what is described for *C. mastitidis* where meso-DPM is also present [8]. The following polar lipids were detected: diphosphatidylglycerol, phosphatidylglycerol, aminolipid, aminoglycolipid, glycolipid, glycophospholipid, phospholipid and unidentified lipid. The major fatty acids were $C_{16:0}$ (24.4%), $C_{18:1}\omega9c$ (23.7%), $C_{18:0}$ ante/$C_{18:2}\omega6,9c$ (23.6%) and $C_{18:0}$ (11.1%) (Supplementary Table 1). These results are similar to what is described for *C. mastitidis* ($C_{16:0}$ (24%), $C_{18:1}\omega9c$ (29%) $C_{18:0}$ (24%)), *C. lowii* ($C_{16:0}$ (30%), $C_{18:1}\omega9c$ (34%) $C_{18:0}$ (19%)) and *C. oculi* ($C_{16:0}$ (29%), $C_{18:1}\omega9c$ (33 %), $C_{18:0}$ (17%) [4].

Physiology

For all six strains growth was observed aerobically at 18 °C, 25 °C, 30 °C and 37 °C and anaerobically (tested at 37 °C). At 18 °C colonies were only visible after 3 days of incubation while at 25–37 °C pinpoint colonies were observed after 1 day. At 44 °C growth was largely inhibited. Growth was optimal at 30–37 °C and enhanced by 5% CO_2 atmosphere. All strains showed some measure of dissociative growth with smaller and larger colonies.

The proposed species was found to be fastidious requiring enriched media such as TSA SB agar, Thioglycollate Medium enriched with vitamin K_1 and hemin (BD) or Tryptone Soya Broth (Oxoid). Growth was not observed by eye in Cation adjusted Mueller Hinton Broth (Thermo Scientific) and on Brolac agar (Oxoid) after 5 days of incubation at 37 °C.

The CAMP-test with *Staphylococcus aureus* was negative for all strains. The esculin test using esculin hydrolysis discs was positive for all strains while it was negative for *C. frankenforstense* DSM 45800^T, *C. mastitidis* DSM 44356^T, *C. lowii* DSM 107388^T, *C. oculi* DSM 107387^T, *C. renale* DSM 20688^T and *C. bovis* DSM 20582^T. Results obtained using the VITEK® 2 ANC card are shown in Table 4. Strains 18M0132^T, 17M2518, 18M0913, 19M0083, 20M1046 and 20M1090 were positive for leucine arylamidase, Ellman's reagent, phenylalanine arylamidase, L-proline arylamidase, L-pyrrolidonyl arylamidase, D-glucose, sucrose and urease. They were negative for D-galactose, D-cellobiose, tyrosine arylamidase, Ala-Phe-Pro arylamidase, D-maltose, arbutin, N-acetyl-D-glucosamine, beta-galactopyranosidase indoxyl, alpha-arabinosidase, 5-bromo-4-chloro-3-indoxyl-alpha-galactoside, beta-mannosidase, maltotriose, beta-D-fucosidase, 5-bromo-4-chloro-3-indoxyl-beta-N-acetyl-glucosamide, 5-bromo-4-chloro-3-indoxyl-alpha-mannoside, alpha-L-fucosidase, phosphatase, L-arabinose, D-ribose 2, phenylphosphonate, alpha-L-arabinfuranoside, D-xylose. They were variable for D-mannose (type strain positive), 5-bromo-4-chloro-3-indoxyl-beta-glucoside (type strain negative), 5-bromo-4-chloro-3-indox

Table 4
Biochemical profiles of strains investigated in this study based on VITEK® 2 ANC card. Weak reactions (slightly below or above the threshold) are indicated in brackets. 1: 18M0132^T, 2: 17M2518, 3: 18M0913, 4: 19M0083, 5: 20M1046, 6: 20M1090, 7: *C. frankenforstense* DSM 45800^T, 8: *C. mastitidis* DSM 44356^T, 9: *C. lowii* DSM 107388^T, 10: *C. oculi* DSM 107387^T 11: *C. bovis* DSM 20582^T, 12: *C. renale* DSM 20688^T.

	1	2	3	4	5	6	7	8	9	10	11	12
D-galactose	-	-	-	-	-	-	-	-	-	-	-	-
Leucin-arylamidase	+	+	+	+	+	+	+	+	+	+	+	+
Ellman's reagent	+	+	+	+	+	+	+	-	+	-	+	+
Phenylalanine arylamidase	+	+	+	+	+	+	+	+	+	+	+	+
L-proline arylamidase	+	+	+	+	+	+	+	+	+	+	+	+
L-pyrrolidonyl-arylamidase	+	+	+	+	+	+	-	-	+	-	-	-
D-cellobiose	-	-	-	-	-	-	-	-	-	-	-	-
Tyrosine arylamidase	-	-	-	(-)	-	-	+	+	+	+	+	-
Ala-Phe-Pro-arylamidase	-	-	-	-	-	-	-	(-)	+	-	-	-
D-glucose	+	+	+	+	+	+	+	-	-	+	+	+
D-mannose	+	+	+	+	+	-	+	-	-	(-)	-	+
D-maltose	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+	+	-	-	-	-	-
Arbutin	-	-	-	-	-	-	-	-	-	-	-	-
N-acetyl-D-glucosamine	-	-	-	-	-	-	-	-	-	-	-	-
5-bromo-4-chloro-3-indoxyl-beta-glucoside	(-)	-	-	+	-	-	-	-	-	-	-	-
Urease	+	+	+	+	+	+	-	+	+	+	+	+
5-bromo-4-chloro-3-indoxyl-beta-glucuronide	-	-	+	-	+	-	+	-	-	-	-	+
Beta-galactopyranosidase indoxyl	-	-	-	-	-	-	-	-	-	-	+	-
Alpha-arabinosidase	-	-	-	-	-	-	-	-	-	-	-	-
5-bromo-4-chloro-3-indoxyl-alpha-galactoside	-	-	-	-	-	-	-	-	-	-	-	-
Beta-mannosidase	-	-	-	-	-	-	-	-	-	-	-	-
Arginin-GP	-	-	+	-	-	-	-	-	(-)	+	-	-
Pyruvate	(+)	-	+	(+)	+	-	+	-	+	-	+	+
Maltotriose	-	-	-	-	-	-	-	-	-	-	-	-
Esculin-hydrolysis	+	+	-	+	-	+	-	-	-	-	-	-
Beta-D-fucosidase	-	-	-	-	-	-	-	-	-	-	-	-
5-bromo-4-chloro-3-indoxyl-beta-N-acetyl-glucosamide	-	-	-	-	-	-	-	-	-	-	-	-
5-bromo-4-chloro-3-indoxyl-alpha-mannoside	-	-	-	-	-	-	-	-	-	-	-	-
Alpha-L-fucosidase	-	-	-	-	-	-	-	-	-	-	-	-
Phosphatase	-	-	-	-	-	-	-	-	(+)	-	-	-
L-arabinose	-	-	-	-	-	-	-	-	-	-	-	-
D-ribose 2	-	-	-	-	-	-	+	-	-	+	-	(+)
Phenylphosphonate	-	-	-	-	-	-	-	-	-	-	-	-
Alpha-L-arabinfuranoside	-	-	-	-	-	-	-	-	-	-	-	-
D-xylose	-	-	-	-	-	-	-	-	-	-	-	-

Table 5
Minimal inhibitory concentrations for strains 18M0132^T, 17M2518, 18M0913, 19M0083, 20M1046 and 20M1090. Breakpoints according to CLSI guidelines for *Corynebacterium* spp. and Coryneforms [30]. BP = breakpoint, NA = not available.

Antimicrobial agent	BP (S<=)	Test range (µg/ml)	MIC µg/ml per strain					
			18M0132^T	17M2518	18M0913	19M0083	20M1046	20M1090
Gentamicin	4	0.12–256	<=0.12	<=0.12	<=0.12	<=0.12	<=0.12	<=0.12
Ciprofloxacin	NA	0.008–16	0.12	0.12	0.12	0.12	0.12	0.12
Enrofloxacin	NA	0.008–16	0.12	0.12	0.12	0.12	0.12	0.12
Marbofloxacin		0.008–16	0.25	0.25	0.5	0.25	0.25	0.25
Trimethoprim/ sulfamethoxazole	2/38	0.015/0.29–32/608	2/38	4/76	4/76	4/76	4/76	4/76
Tetracycline	4	0.12–356	0.25	0.5	0.5	0.5	0.5	0.25
Tilmicosin	NA	0.06–128	0.5	0.5	0.5	0.5	0.5	0.5
Tulathromycin	NA	0.06–32	0.5	0.25	0.25	0.5	0.5	0.25
Ampicillin	NA	0.03–64	0.25	0.25	0.25	0.25	0.12	0.25
Amoxicillin/ clavulanic acid	NA	0.03/0.015–64/32	0.06/0.03	0.12/0.06	0.12/0.06	0.12/0.06	0.06/0.03	0.06/0.03
Penicillin	0.12	0.015–32	0.12	0.12	0.06	0.12	0.03	0.06
Cephalothin	NA	0.06–128	<=0.06	<=0.06	0.12	0.12	<=0.06	<=0.06
Cefoperazone	NA	0.06–32	2	2	1	2	1	1
Cefotaxime	NA	0.015–32	0.5	0.5	0.5	0.5	0.25	0.5
Ceftiofur	NA	0.03–64	1	1	1	1	0.5	1
Cefquinome	NA	0.015–32	0.25	0.5	0.5	0.5	0.25	0.25

yl-beta-glucuronide (type strain negative), arginine- GP (type strain negative), pyruvate (type strain positive), esculin hydrolysis using the VITEK® 2 system (type strain positive).

Antimicrobial resistance testing revealed similar MICs for all tested antimicrobials of the six strains (Table 5). They can all be considered susceptible to gentamicin, penicillin, and tetracycline following CLSI veterinary breakpoints for corynebacteria [30]. The MIC for trimethoprim / sulfamethoxazole was just above the breakpoint for five of the six strains and for one just below. Since this is not a species specific breakpoint the result has to be interpreted with caution and likely indicates the wild type. This is also in line with the absence of known resistance genes in the genomes.

Conclusion

Based on biochemical, chemotaxonomic and phylogenetic, analyses strains 18M0132^T, 17M2518, 18M0913, 19M0083, 20M1046 and 20M1090 clearly belong to a single novel species of the genus

Table 6
Description of *Corynebacterium uberis* sp. nov.

Genus name	*Corynebacterium*
Species name	*Corynebacterium uberis*
Specific epithet	*uberis*
Species status	sp. nov.
Species etymology	L. gen. n. uberis, of an udder
Description of the new taxon and diagnostic traits	The bacteria are Gram-positive non-motile rods. Catalase reaction is positive. Colonies on sheep blood agar are about 1 mm after 72 h aerobic incubation at 37 °C. They are non-hemolytic, greyish white and show some degree of dissociative growth. *C. uberis* sp. nov. is CAMP-test negative. It is positive for leucine arylamidase, Ellman's reagent, phenylalanine arylamidase, L-proline arylamidase, L-pyrrolidonyl arylamidase, D-glucose, sucrose and urease. *C. uberis* sp. nov. is negative for D-galactose, D-cellobiose, tyrosine arylamidase, Ala-Phe-Pro-arylamidase, D-maltose, arbutin, N-acetyl-D-glucosamine, beta-galactopyranosidase indoxyl, alpha-arabinosidase, 5-bromo-4-chloro-3-indoxyl-alpha-galactoside, beta-mannosidase, maltotriose, beta-D-fucosidase, 5-bromo-4-chloro-3-indoxyl-beta-N-acetyl-glucosamide, 5-bromo-4-chloro-3-indoxyl-alpha-mannoside, alpha-L-fucosidase, phosphatase, L-arabinose, D-ribose 2, phenylphosphonate, alpha-L-arabinfuranoside, D-xylose. It is variable for D-mannose, 5-bromo-4-chloro-3-indoxyl-beta-glucoside, 5-bromo-4-chloro-3-indoxyl-beta-glucuronide, arginine- GP, pyruvate. Esculin hydrolysis is positive using esculin hydrolysis discs. The main menaquinones are MK-8(H_2) (43.8%) and MK-9(H_2) (45.5%). No mycolic acids were detected. The peptidoglycan type of *Corynebacterium uberis* sp. nov. is A1γ meso-DPM – direct.The major fatty acids were $C_{16:0}$ (24.4%), $C_{18:1}$ω9c (23.7%), $C_{18:0}$ ante/$C_{18:2}$ω6,9c (23.6%) and $C_{18:0}$ (11.1%).
Country of origin	Switzerland
Region of origin	Bern
Date of isolation (dd/mm/yyyy)	24/01/2018
Source of isolation	Bovine mastitis milk
Sampling date (dd/mm/yyyy)	22/01/2018
Latitude (xx°xx′xx″N/S)	46.95700° North
Longitude (xx°xx′xx″E/W)	7.42600° East
Altitude (meters above sea level)	566
16S rRNA gene accession nr.	Genomes
Genome accession number	GenBank CP085051.1
Genome status	Complete
Genome size	2,533 kbp
GC mol%	65.5
Number of strains in study	6
Source of isolation of non-type strains	Bovine mastitic milk
Information related to the Nagoya Protocol	Country of origin: Switzerland
Designation of the Type Strain	18M0132^T
Strain Collection Numbers	Strain 18M0132^T was deposited in the Culture Collection of Switzerland (accession number: CCOS 1972^T) and the German Collection of Microorganisms and Cell Cultures DSMZ (accession number: DSM 111922^T)

Corynebacterium for which the name *Corynebacterium uberis* sp. nov is proposed (Table 6). *Corynebacterium uberis* sp. nov. has repeatedly been isolated from bovine mastitis milk indicating a role as opportunistic pathogen, which should be investigated in future studies.

Repositories

Strain 18M0132^T was deposited in the Culture Collection of Switzerland (accession number: CCOS 1972^T) and the German Collection of Microorganisms and Cell Cultures DSMZ (accession number: DSM 111922^T).

Complete genome sequences of strains 18M0132^T (CP085051.1), 17M2518 (CP085050.1), 18M0913 (CP085049.1), 19M0083 (CP085048.1), 20M1046 (CP085047.1) and 20M1090 (CP085046.1) were deposited in GenBank (PRJNA769678).

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Consent for publication

Not applicable.

CRediT authorship contribution statement

Sonja Kittl: Conceptualization, Formal analysis, Investigation, Writing - original draft, Writing-review and editing. **Eveline Studer:** Resources, Writing-review and editing. **Isabelle Brodard:** Investigation, Writing-review and editing. **Andreas Thomann:** Investigation, Writing-review and editing. **Jörg Jores:** Conceptualization, Writing-review and editing

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.syapm.2022.126325.

References

[1] Nouioui, I., Carro, L., García-López, M., Meier-Kolthoff, J.P., Woyke, T., Kyrpides, N.C., Pukall, R., Klenk, H.P., Goodfellow, M., Goker, M. (2018) Genome-based taxonomic classification of the Phylum *Actinobacteria*. Front Microbiol 9, 2007. https://doi.org/10.3389/fmicb.2018.02007.

[2] Baek, I., Kim, M., Lee, I., Na, S.I., Goodfellow, M., Chun, J. (2018) Phylogeny trumps chemotaxonomy: a case study involving *Turicella otitidis*. Front Microbiol 9, 834. https://doi.org/10.3389/fmicb.2018.00834.

[3] A. Tauch A. Burkovski Molecular armory or niche factors: virulence determinants of Corynebacterium species fnv185.

[4] Bernard, K.A., Pacheco, A.L., Loomer, C., Burdz, T., Wiebe, D., Huynh, C., Kaplen, B., Olson, A.B., Cnockaert, M., Eguchi, H., Kuwahara, T., Nakayama-Imaohji, H., Shiota, H., Boudewijns, M., Van Hoecke, F., Vandamme, P. (2016) *Corynebacterium lowii* sp. nov. and *Corynebacterium oculi* sp. nov., derived from human clinical disease and an emended description of *Corynebacterium mastitidis*. Int J Syst Evol Microbiol 66, 2803–2812. https://doi.org/10.1099/ijsem.0.001059.

[5] Markey, B.K. Clinical veterinary microbiology. Elsevier, Edinburgh.

[6] Lücken, A., Wente, N., Zhang, Y., Woudstra, S., Krömker, V. (2021) Corynebacteria in bovine quarter milk samples-species and somatic cell counts. Pathogens 10 (7), 831.

[7] J.L. Goncalves, T. Tomazi, J.R. Barreiro, D.C. Beuron, M.A. Arcari, S.H. Lee, C.M. Martins, J.P. Araujo Junior, M.V. dos Santos, Effects of bovine subclinical mastitis caused by *Corynebacterium* spp. on somatic cell count, milk yield and composition by comparing contralateral quarters, Vet J, 209 (2016) 87-92, https://doi.org/10.1016/j.tvjl.2015.08.009.

[8] Fernandez-Garayzabal, J.F., Collins, M.D., Hutson, R.A., Fernandez, E., Monasterio, R., Marco, J., Dominguez, L. (1997) *Corynebacterium mastitidis* sp. nov., isolated from milk of sheep with subclinical mastitis. Int J Syst Bacteriol 47, 1082–1085. https://doi.org/10.1099/00207713-47-4-1082.

[9] A.J. St Leger, J.V. Desai, R.A. Drummond, A. Kugadas, F. Almaghrabi, P. Silver, K. Raychaudhuri, M. Gadjeva, Y. Iwakura, M.S. Lionakis, R.R. Caspi, An ocular commensal protects against corneal infection by driving an interleukin-17 response from Mucosal gammadelta T cells, Immunity, 47 (2017) 148-158 e145, https://doi.org/10.1016/j.immuni.2017.06.014.

[10] Aoki, T., Kitazawa, K., Deguchi, H., Sotozono, C. (2021) Current evidence for *Corynebacterium* on the ocular surface. Microorganisms 9, 254.

[11] Pitcher, D.G., Saunders, N.A., Owen, R.J. (1989) Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett Appl Microbiol 8, 151–156. https://doi.org/10.1111/j.1472-765X.1989.tb00262.x.

[12] Kolmogorov, M., Yuan, J., Lin, Y., Pevzner, P.A. (2019) Assembly of long, error-prone reads using repeat graphs. Nat Biotechnol 37, 540–546. https://doi.org/10.1038/s41587-019-0072-8.

[13] Hunt, M., Silva, N.D., Otto, T.D., Parkhill, J., Keane, J.A., Harris, S.R. (2015) Circlator: automated circularization of genome assemblies using long sequencing reads. Genome Biol 16, 294. https://doi.org/10.1186/s13059-015-0849-0.

[14] Chun, J., Oren, A., Ventosa, A., Christensen, H., Arahal, D.R., da Costa, M.S., Rooney, A.P., Yi, H., Xu, X.W., De Meyer, S., Trujillo, M.E. (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68, 461–466. https://doi.org/10.1099/ijsem.0.002516.

[15] Kuhnert, P., Capaul, S.E., Nicolet, J., Frey, J. (1996) Phylogenetic positions of *Clostridium chauvoei* and *Clostridium septicum* based on 16S rRNA gene sequences. Int J Syst Bacteriol 46, 1174–1176. https://doi.org/10.1099/00207713-46-4-1174.

[16] Yoon, S.H., Ha, S.M., Kwon, S., Lim, J., Kim, Y., Seo, H., Chun, J. (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 67, 1613–1617. https://doi.org/10.1099/ijsem.0.001755.

[17] Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., Battistuzzi, F.U. (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35 (6), 1547–1549.

[18] Meier-Kolthoff, J.P., Auch, A.F., Klenk, H.P., Göker, M. (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14, 60. https://doi.org/10.1186/1471-2105-14-60.

[19] Meier-Kolthoff, J.P., Göker, M. (2019) TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10, 2182. https://doi.org/10.1038/s41467-019-10210-3.

[20] Emms, D.M., Kelly, S. (2019) OrthoFinder: phylogenetic orthology inference for comparative genomics. Genome Biol 20, 238. https://doi.org/10.1186/s13059-019-1832-y.

[21] D.M. Emms, S. Kelly, STAG: Species Tree Inference from All Genes, https://doi.org/10.1101/267914.

[22] Emms, D.M., Kelly, S. (2017) STRIDE: Species tree root inference from gene duplication events. Mol Biol Evol 34, 3267–3278. https://doi.org/10.1093/molbev/msx259.

[23] Emms, D.M., Kelly, S. (2015) OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. Genome Biol 16, 157. https://doi.org/10.1186/s13059-015-0721-2.

[24] Seemann, T. (2014) Prokka: rapid prokaryotic genome annotation. Bioinformatics 30, 2068–2069. https://doi.org/10.1093/bioinformatics/btu153.

[25] Na, S.I., Kim, Y.O., Yoon, S.H., Ha, S.M., Baek, I., Chun, J. (2018) UBCG: Up-to-date bacterial core gene set and pipeline for phylogenomic tree reconstruction. J Microbiol 56, 280–285. https://doi.org/10.1007/s12275-018-8014-6.

[26] Lee, I., Ouk Kim, Y., Park, S.C., Chun, J. (2016) OrthoANI: An improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 66, 1100–1103. https://doi.org/10.1099/ijsem.0.000760.

[27] Silva, M., Machado, M.P., Silva, D.N., Rossi, M., Moran-Gilad, J., Santos, S., Ramirez, M., Carrico, J.A. (2018) chewBBACA: A complete suite for gene-by-

gene schema creation and strain identification. Microb Genom 4. https://doi.org/10.1099/mgen.0.000166.
[28] Liu, B., Zheng, D., Zhou, S., Chen, L., Yang, J. (2022) VFDB 2022: A general classification scheme for bacterial virulence factors. Nucl Acids Res 50, D912–D917. https://doi.org/10.1093/nar/gkab1107.
[29] Schumann, P. (2011) Peptidoglycan structure. Method Microbiol 38, 101–129. https://doi.org/10.1016/B978-0-12-387730-7.00005-X.
[30] CLSI, Methods for antimicrobial susceptibility testing of infrequently isolated or fastidious bacteria isolated from animals CLSI supplement VET06 1st ed. 2017 Clinical and Laboratory Standards Institute.
[31] Chun, J., Lee, J.H., Jung, Y., Kim, M., Kim, S., Kim, B.K., Lim, Y.W. (2007) EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. Int J Syst Evol Microbiol 57, 2259–2261. https://doi.org/10.1099/ijs.0.64915-0.
[32] Goris, J., Konstantinidis, K.T., Klappenbach, J.A., Coenye, T., Vandamme, P., Tiedje, J.M. (2007) DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57, 81–91. https://doi.org/10.1099/ijs.0.64483-0.
[33] Rossello-Mora, R., Amann, R. (2015) Past and future species definitions for bacteria and archaea. Syst Appl Microbiol 38, 209–216. https://doi.org/10.1016/j.syapm.2015.02.001.