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TAXONOMIC DESCRIPTION
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Aquirufa ecclesiirivi sp. nov. and *Aquirufa beregesia* sp. nov., isolated from a small creek and classification of *Allopseudarcicella aquatilis* as a later heterotypic synonym of *Aquirufa nivalisilvae*

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Abstract

Two bacterial strains, 50A-KIRBA^T and 50C-KIRBA^T, were isolated from the same freshwater creek located near Salzburg, Austria. They showed 16S rRNA gene sequence similarities to *Aquirufa nivalisilvae* of 100 and 99.9%, respectively. A genome-based phylogenetic reconstruction with amino acid sequences of 119 single-copy genes suggested that the new strains represent two new species of the genus *Aquirufa*. Pairwise calculated whole-genome average nucleotide identity (gANI) values ranging from 85.4 to 87.5% confirmed this conclusion. Phenotypic, chemotaxonomic and genomic traits were investigated. Like strains of other *Aquirufa* species, 50A-KIRBA^T and 50C-KIRBA^T grew aerobically and chemoorganotrophically, were rod-shaped, red-pigmented and motile, most likely by gliding. They could be distinguished by slight differences in the chemotaxonomic features. We propose to establish for strain 50A-KIRBA^T (=CIP 111735^T=LMG 31080^T) as type strain the name *Aquirufa ecclesiirivi* and for strain 50C-KIRBA^T (=CIP 111736^T=LMG 31501^T) as type strain the name *Aquirufa beregesia*. Furthermore, the relationship between the type strains of *Aquirufa nivalisilvae* (59G-WUEMPEL^T) and *Allopseudarcicella aquatilis* (HME7025^T) was investigated. Results of polyphasic analyses, especially a gANI value of 97.6%, as well as the genome-based phylogenetic reconstruction, suggested that *Allopseudarcicella aquatilis* is a heterotypic synonym of *Aquirufa nivalisilvae*. According to rule 24b of the International Code of Nomenclature of Prokaryotes we propose to classify strain HME7025 as *Aquirufa nivalisilvae* and provide an emended description for the latter.

The genus *Aquirufa*, which belongs to the family *Cytophagaceae* (*Bacteroidetes*), was first described in 2019 by Pitt *et al.* [1] on the basis of three isolates, which originated from small freshwater habitats in Austria and represented two new species. *Aquirufa* formed a clade with the genera *Pseudarcicella*, *Arcicella* and *Flectobacillus* but differed from them by approximately half as large genome sizes. Later, *Sandaracinomonas limnophila* gen. nov., sp. nov. was described [2], which is closely related to *Aquirufa* and of comparable genome size. Two months after the publication of the genus *Aquirufa*, the description of *Allopseudarcicella aquatilis* gen. nov., sp. nov. [3] based on a strain isolated from a stream in Seoul (Republic of Korea) was released. According to polyphasic analyses in this study, the new taxon seemed to be a later heterotypic synonym of *Aquirufa nivalisilvae*. Recently (May 2020), a third *Aquirufa* species was published [4].

Members of *Aquirufa* appear to be widespread and sometimes abundant [5] freshwater bacteria, occurring in various standing and running waters [1]. They are aerobic, chemoorganotrophic, rod-shaped, red-pigmented and motile, most likely by gliding [1]. During a Citizen Science project (Sparkling Science Program) [1] another two bacterial strains were isolated, which represent two new species of the genus *Aquirufa*. We propose the names *Aquirufa ecclesiirivi* for strain 50A-KIRBA^T and *Aquirufa beregesia* for strain 50C-KIRBA^T. Furthermore, we propose to classify *Allopseudarcicella aquatilis* as a later heterotypic synonym of *Aquirufa nivalisilvae*.

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Abbreviations: gANI, whole genome average nucleotide identity; NSY, nutrient broth–soytone–yeast extract; R2A, Reasoner's 2A.
The Whole Genome Shotgun projects and 16S rRNA gene sequences have been deposited at DDBJ/ENA/GenBank. The accession numbers of strain 50A-KIRBA^T are JAANOP000000000 and MT140363; for strain 50C-KIRBA^T they are SEWW00000000 and MK449346, respectively.
One supplementary figure is available with the online version of this article.

HOME HABITAT, ISOLATION AND ECOLOGY

Strains 50A-KIRBA^T and 50C-KIRBA^T were both obtained from a single water sample from Kirchstaettbach, a freshwater creek running through Obertrum, a small town located near Salzburg in Austria. The approximate geographic coordinates of the sampling site are 47.938 N and 13.068 E. Water sampled in April 2018 had a pH of 6.7 and a conductivity of 721 µS cm^{-1}. The water sample was filtered through a 0.65 µm pore size filter and the filtrate subsequently plated on agar plates. For the first cultivation step, for strain 50A-KIRBA^T, nutrient broth–soytone–yeast extract (NSY) medium [6] was used and for strain 50C-KIRBA^T, tryptone–soytone medium [7] was used, which compared to the NSY medium contained lower nutrient concentrations Both strains were purified using NSY liquid medium and agar plates, however, they grew as well with Reasoner's 2A [8] medium.

Looking at BLAST results, obtained by using 16S rRNA gene sequences, the cluster comprising the genera *Aquirufa* and *Sandaracinomonas* seemed to represent widespread freshwater bacteria. However, this study and additional unpublished data suggested a cryptic species complex with comparatively high 16S rRNA gene sequence similarities between the taxa. Especially 16S rRNA gene sequence similarities of 100% or nearly 100% among distinct species (see below), made an appropriate assignment of environmental sequences or sequences from isolates to species impossible. So, it remains unclear if the two new species described here were detected in other habitats. Furthermore, it is uncertain if they are small running water specialists or if they occur in other types of freshwater habitats too.

PHENOTYPIC AND CHEMOTAXONOMIC CHARACTERIZATION

The temperature range for growth was tested on NSY agar plates exposed to different temperatures. The lowest tested temperature was 5 °C. At temperatures close to the maximum temperature of growth, experiments were performed in 1 °C intervals until no growth was observed. All further tests were performed at room temperature (21 °C). NaCl tolerance was tested using NSY agar plates with various NaCl concentrations in 0.1% (w/v) steps. For testing anaerobic growth, an anaerobic chamber and standard NSY agar plates as well as plates supplemented with 2 g l^{-1} $NaNO_3$ were used. For determination of cell morphology and cell dimensions, well-growing liquid cultures were fixed with 2% formaldehyde, stained with DAPI (4′,6-diamidine-2′-phenylindole dihydrochloride) and investigated by using a UV filter under an epifluorescence microscope. Motility was tested using soft agar plates (1 g l^{-1} yeast extract, 0.1 g l^{-1} K_2HPO_4, 2.0 g l^{-1} agar) [9]. One drop of a well-growing culture was placed in the centre of these test plates, as well as on standard NSY plates and observed for several days. Assimilation of various substrates was tested using GEN III MicroPlates (Biolog), which detect utilization of substrates as electron donors by the subsequent reduction of a tetrazolium redox dye. Cells from well-growing liquid cultures were centrifuged (5000 g) and added to the inoculum medium so that the OD of the culture correspond to 0.07 at a wavelength of 590 nm. The absorption was measured with Multiskan FC apparatus (Thermo Scientific) at a wavelength of 595 nm after 48 h incubation at 20 °C. After subtracting the value of the negative control (without substrate), obtained values ranging from 0.016 to 0.05 were regarded as weak utilization and for >0.05 as positive. The chemotaxonomic characterization of the strains included analyses of the composition of whole-cell fatty acids, polar lipids and respiratory quinones. They were carried out by the Identification Service, Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany. For all chemotaxonomic analyses, cells were inoculated to liquid NSY medium and harvested after 3 days of growth at room temperature (21 °C) by centrifugation. For the whole-cell fatty acid composition an Agilent Technologies 6890 N instrument and the Microbial Identification System (MIDI) Sherlock version 6.1 (results were evaluated against the TSBA 40 peak-naming table database) was used as described by Sasser [10]. Polar lipids and respiratory quinones were extracted and analysed as described by Tindall [11, 12] based on the method by Bligh and Dyer [13]. To examine for flexirubin, tests with 20% KOH and 12 M HCl were performed [14]. Results were negative for both strains. The results of the phenotypic and chemotaxonomic investigations compared with other *Aquirufa* strains are given in Table 1. The patterns of the polar lipids are shown in Fig. S1 (available in the online version of this article). The characteristics of strains belonging to the genus *Aquirufa* are relatively similar to each other. Greater differences were observed in the substrate assimilation and polar lipid patterns (Table 1).

GENOMIC CHARACTERIZATION

DNA extraction and genome sequencing were performed as described previously [15]. In the case of strain 50A-KIRBA^T, a shotgun library was paired-end sequenced on an Illumina HiSeq instrument (2×150 bp). *De novo* assemblies were performed by using the software SPAdes version 3.13.1 [16]. This resulted in 41 scaffolds, the nucleotide coverage was 800× and the N50 value was 0.34 Mbp. In the case of strain 50C-KIRBA^T, a shotgun library was paired-end sequenced on an Illumina MiSeq instrument (2×300 bp). *De novo* assemblies were performed by using the software SPAdes version 3.13.0 [16]. This resulted in 91 scaffolds, the nucleotide coverage was 225× and the N50 value was 0.34 Mbp. The obtained genome sequences were annotated by the NCBI Prokaryotic Genome Annotation Pipeline and for further analyses by the Integrated Microbial Genomes and Microbiomes Expert Review (IMG/MER) [17] system. The IMG/MER Genome IDs are 2828879446 (strain 50A-KIRBA^T) and 2816332124 (strain 50C-KIRBA^T), for the other strains see [1].

Using the 'Genome Gene Best Homologs' tool of the IMG/MER system [17], which find corresponding genes between two genomes, strain 50A-KIRBA^T (2719 genes) and strain 50C-KIRBA^T (2784 genes) shared on average 88% of their predicted genes with sequence identities of more than 60%.

Table 1. Comparison of phenotypic and chemotaxonomic traits of the new strains and related taxa

Strain: 1, 50A-KIRBA^T; 2, 50C-KIRBA^T; 3, *Aquirufa antheringensis* 30S-ANTBAC^T; 4, *Aquirufa nivalisilvae* 59G-WUEMPEL^T; 5, *Allopseudarcicella aquatilis* HME7025^T. Only fatty acids with values ≥1% for at least one of the strains are listed. For assimilation patterns, only positive results for at least one strain are shown; the rest of the substrates in the GEN III MicroPlates (Biolog) gave negative results (see species descriptions). Data in columns 3 and 4 are published elsewhere [1], but were evaluated in the same lab under the same conditions. Data in column 5 for fatty acids, polar lipids and respiratory quinones are taken from [3]. +, Positive; –, negative/not detected; w, weak; tr, trace.

	1	2	3	4	5
Cell morphology	Rods	Rods	Rods	Rods	Rods
Mean cell length (μm)	1.7	1.5	1.7	1.6	1.2
Mean cell width (μm)	0.5	0.3	0.6	0.5	0.4
Temperature range for growth (°C)	5–34	5–30 (w)	5–32 (w)	5–35 (w)	5–36 (w)
NaCl tolerance (%, w/v)	0–0.4	0–0.2 (w)	0–0.3 (w)	0–0.4	0–0.3
Anaerobic growth	–	–	–	–	–/+*
Motility on soft agar	+	+	+	+	+
Pigmentation	Red	Red	Red	Red	Red
Assimilation of:					
Pectin	–	+	+	+	–
Tween 40	w	+	+	+	+
Acetoacetic acid	w	w	w	w	–
Glucoronamide	–	w	w	w	w
D-Fructose-6-PO_4	–	w	w	w	–
Sucrose	–	–	–	w	w
D-Mannose	–	–	–	w	–
D-Salicin	–	–	–	w	–
D-Aspartic acid	–	–	–	w	–
α-D-Glucose	–	–	–	–	w
Dextrin	w	–	–	–	w
D-Arabitol	–	–	–	–	w
N-Acetyl-D-glucosamine	–	–	–	–	w
N-Acetyl-D-galactosamine	–	–	–	–	w
Acetic acid	–	w	–	–	–
Fatty acids:					
$C_{14:0}$	1.0	0.6	2.9	0.4	1.0
$C_{15:0}$	–	2.2	2.2	–	–
$C_{15:1}$ ω6*c*	0.7	2.5	1.5	1.1	–
$C_{16:1}$ ω5*c*	13.9	6.7	11.9	3.0	7.8
$C_{17:1}$ ω6*c*	1.2	5.4	1.1	1.4	–
iso-$C_{11:0}$	1.7	1.3	3.6	1.2	1.2
iso-$C_{13:0}$	0.6	0.5	0.8	1.4	1.4
iso-$C_{15:0}$	24.5	22.7	20.3	34.4	41.9
anteiso-$C_{15:0}$	8.4	8.3	5.6	10.7	10.8

Continued

Table 1. Continued

	1	2	3	4	5
iso-$C_{15:0}$ 3-OH	9.3	8.3	13.4	10.5	8.1
iso-$C_{17:0}$ 3-OH	2.1	3.1	1.1	3.3	2.8
iso-$C_{17:1}$ ω9*c*	1.2	2.9	0.4	2.0	3.2**
Summed feature 2†	0.6	0.4	1.3	0.2	TR
Summed feature 3†	21.0	21.8	24.7	16.8	12.2
Summed feature 4†	2.7	2.8	1.1	3.0	2.9
unknown 14.959	4.6	5.0	2.4	5.0	–
Polar lipids:					
Phosphatidylethanolamine	+	+	+	+	+
Unidentified aminolipids	–	1	–	1	1
Unidentified aminophospholipids	3	3	2	3	3
Unidentified phosholipids	–	1	–	–	1
Unidentified polar lipids	2	2	4	5	4
Unidentified glycolipids	–	–	–	–	2
Respiratory quinones:					
MK7	+	+	+	+	+
MK6	–	TR	TR	–	TR

*Weak anaerobic growth on NSY plates without added nitrate.
†Summed features represent groups of fatty acids which could not be separated by GLC and the MIDI system. Summed feature 2, $C_{12:0}$ aldehyde, unknown 10.928, iso-$C_{16:1}$ I, $C_{14:0}$ 3-OH; summed feature 3, $C_{16:1}$ *ω7c*, $C_{16:1}$ *ω6c*, iso-$C_{15:0}$ 2-OH; summed feature 4, iso-$C_{17:1}$ I, anteiso-$C_{17:1}$ B/i I; **, and/or 10-methyl $C_{16:0}$.

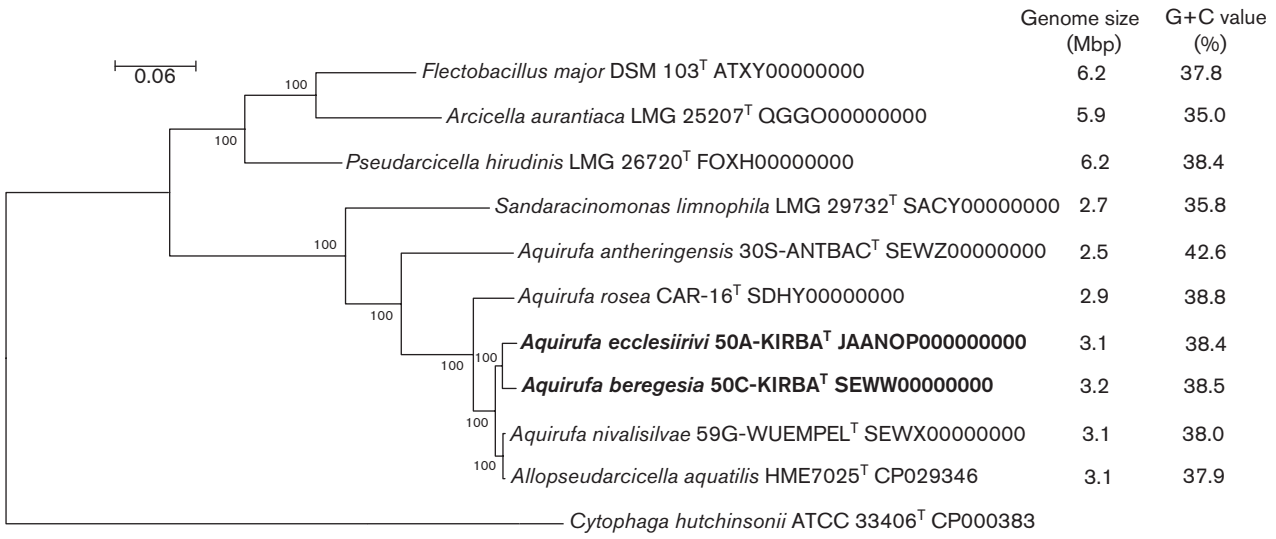

Fig. 1. Phylogenomic RAxML tree calculated with amino acid sequences obtained from 119 single-copy genes from all available genomes of the taxa from Fig. 2. Bar, 0.06 substitutions per nucleotide position.

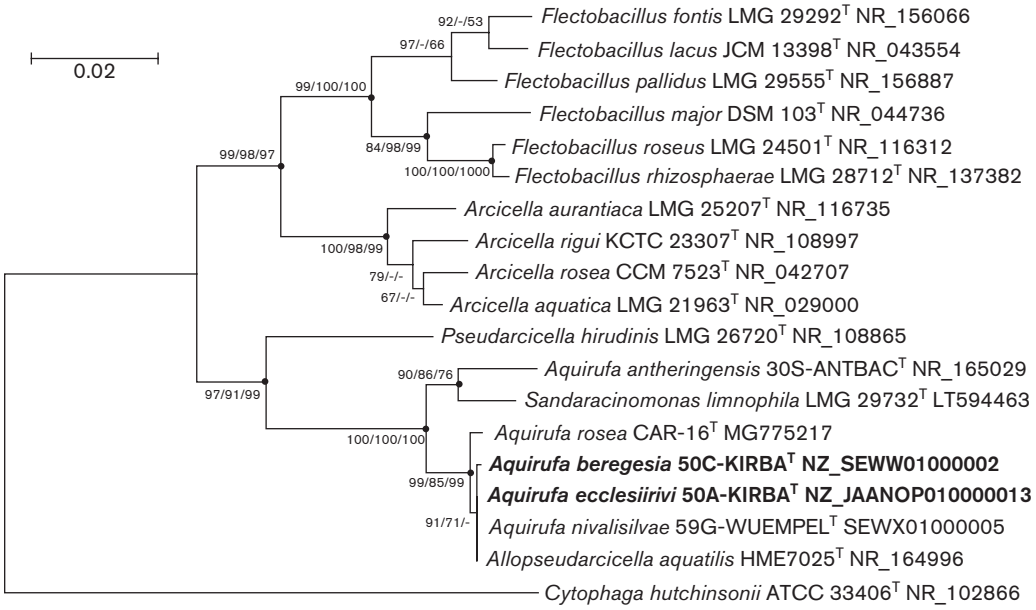

Fig. 2. Reconstruction of the phylogenetic position of the investigated strains based on almost full-length 16S rRNA gene sequences (1350 alignment positions). Shown is the neighbour-joining tree. Bootstrap values are shown from left to right for neighbour-joining, maximum- likelihood and maximum-parsimony trees calculated with the same sequence set. Bar, 0.02 substitutions per nucleotide position. Dots at nodes represent results from all calculated trees.

The percentage of shared genes with the type strain of the nearest relative *Aquirufa nivalisilvae* 59G-WUEMPEL^T (Fig. 1) were 92% for strain 50A-KIRBA^T and 87.5% for strain 50C-KIRBA^T. In contrast, strain 59G-WUEMPEL^T (2674 genes) and strain HME7025^T (2677 genes) shared 96% of their predicted genes.

Both new strains lacked genes putatively encoding for proteorhodopsin and the usually accompanying β-carotene 1,15′-monooxygenase, which are both present in strains belonging to *Aquirufa antheringensis* [1]. Like the other *Aquirufa* strains used for comparison (Table 1), both new strains possessed genes annotated for enzymes necessary for the biosynthesis of various carotenoids (KEGG map00906) and a number of genes putatively associated with gliding motility. Like the other *Aquirufa* strains, with the exception of the type strain of *Aquirufa antheringensis*, strains 50A-KIRBA^T and 50C-KIRBA^T had genes putatively encoding for a nitrous oxide reductase (EC:1.7.2.4).

PHYLOGENY

Phylogenetic trees were calculated by using almost full-length sequences of the 16S rRNA gene (Fig. 2) and by using a concatenated multilocus sequence set consisting of the amino acid sequences of 119 single copy marker genes out of the 120 genes recommended by Parks *et al.* [18] (Fig. 1). For the phylogenetic tree based on 16S rRNA genes (Fig. 2), the sequences were aligned, resulting in a total of 1350 positions in the final dataset, analysed for the best-fitting substitution model and used for reconstruction of three phylogenetic trees, with the parameters of Kimura's two-parameter method [19], gamma distribution (five categories) and 1000 bootstrap replications by using the software MEGA X [20]. For the phylogenomic tree one gene (protein family TIGR00095) of the 120 protein-encoding single copy genes recommended by Parks *et al.* [18] was not present in any of the genomes. The encoded amino acid sequences of the remaining 119 genes were concatenated and aligned by using MAFFT [21]. The software GBLOCKS (version 0.91b) [22] was used to filter out highly variable positions, which resulted in a reduction of the alignment length from 47369 to 41212 positions in 319 selected blocks, which corresponded to 87% of the initial alignment positions. An RAxML tree [23] (Fig. 1) with 100 bootstrap replications was calculated using the CIPRES Science Gateway version 3.3 [24].

The 16S rRNA gene sequence identities between the closest relative *Aquirufa nivalisilvae* and strains 50A-KIRBA^T and 50C-KIRBA^T were 100 and 99.9%, respectively. Furthermore, the sequences of *Aquirufa nivalisilvae* 59G-WUEMPEL^T and *Allopseudarcicella aquatilis* HME7025^T were identical. As reported for other genera [25], the 16S rRNA gene tree could not fully resolve the phylogeny within the branch comprising these four type strains (Fig. 2). In contrast, the genome-based phylogenetic tree, which showed bootstrap values of 100% for all nodes, gave much better insights (Fig. 1). The two new strains were placed on a branch beside *Aquirufa nivalisilvae* and separated from each other (Fig. 1). In addition, the tree revealed the very close relationship between *Aquirufa nivalisilvae*, strain 59G-WUEMPEL^T and *Allopseudarcicella*

aquatilis HME7025^T (Fig. 1). In addition, the genome-based tree showed a separation of *Sandaracidomonas limnophila* from the *Aquirufa* species.

PROPOSAL OF *AQUIRUFA ECCLESIIRIVI* SP. NOV. AND *AQUIRUFA BEREGESIA* SP. NOV.

To test if strains 50A-KIRBA^T and 50C-KIRBA^T represent new species, pairwise whole genome average nucleotide identity (gANI) values were calculated using the IMG/MER system [17]. With the type strain of *Aquirufa nivalisilvae* as their closest relative (Figs. 1 and 2), they showed gANI values of 85.4 and 85.9%, respectively. Comparison of strains 50A-KIRBA^T and 50C-KIRBA^T resulted in a gANI value of 87.5%. All obtained values were clearly lower than the proposed cut-off of 95–96% for separating species [26–29]. The genomic tree based on 119 amino acid sequences (Fig. 1) confirmed these findings (see above). The phenotypic and chemotaxonomic features of the strains belonging to the genus *Aquirufa* were relatively similar (Table 1). Nevertheless, they can be differentiated by a combination of characteristics. Strain 50A-KIRBA^T lacked fatty acid $C_{15:0}$, while relatively high amounts of $C_{16:1}$ ω5*c* were present. In the case of strain 50C-KIRBA^T, fatty acid $C_{15:0}$ and relatively high amounts of $C_{17:1}$ ω6*c* were present (Table 1). The patterns of the polar lipids differed between the two strains and from the other *Aquirufa* strains (Fig. S1; [1]. So, we propose to establish two new species, *Aquirufa ecclesiirivi* sp. nov. and *Aquirufa beregesia* sp. nov., within the genus *Aquirufa*.

PROPOSAL OF CLASSIFICATION OF *ALLOPSEUDARCICELLA AQUATILIS* AS *AQUIRUFA NIVALISILVAE*

The phenotypic and chemotaxonomic characteristics of strain HME7025^T (*Allopseudarcicella aquatilis*) and strain 59G-WUEMPEL^T (*Aquirufa nivalisilvae*) were very similar and differed only in some minor details (Table 1). These features, as well as the 16S rRNA gene sequence similarity of 100%, indicated clearly that the two strains belong to the same genus. Phylogenetic reconstruction with the amino acid sequence of 119 single-copy genes (Fig. 1) suggested that they also belong the same species. To confirm this, the gANI value was calculated using the IMG/MER system [17]. The obtained value of 97.6% was significantly higher than the proposed demarcation of 95–96% between two species [26–29]. So, we concluded that *Allospeudarcicella aquatilis* Kim *et al.* 2019 is a later heterotypic synonym of *Aquirufa nivalisilvae* Pitt *et al.* 2019 and according to rule 24b of the International Code of Nomenclature of Prokaryotes [30] we propose to assign strain HME7025 to the species *Aquirufa nivalisilvae* [1].

DESCRIPTION OF *AQUIRUFA ECCLESIIRIVI* SP. NOV.

Aquirufa ecclesiirivi (ec.cle.si.i.ri'vi. L. fem. n. *ecclesia*, church; L. masc. n. *rivus*, creek; N.L. gen. n. *ecclesiirivi*, from Churchcreek).

Cells form rods, about 1.7 µm long and 0.5 µm wide. Colonies grown on NSY or Reasoner's 2A (R2A) agar are bright red, in older stages dark red, pigmented, circular and convex with smooth surface. Liquid cultures grown in NSY or R2A medium have an intense red-orange colouring. Cells are able to move on soft agar. Growth occurs at 5–34 °C and in 0–0.4% (w) NaCl. Cells weakly assimilate Tween 40, acetoacetic acid and dextrin, and do not assimilate pectin, glucuronamide, D-fructose-6-PO_4, sucrose, D-mannose, D-salicin, D-aspartic acid, L-histidine, propionic acid, D-lactic acid methyl ester, L-alanine, L-glutamic acid, L-aspartic acid, D-glucose-6-PO_4, D-glucuronic acid, D-fructose, D-arabitol, α-D-glucose, α-hydroxy-butyric-acid, D-galactose, methyl β-D-glucoside, D-galacturonic acid, 3-methyl glucose, L-rhamnose, L-galactonic acid lactone, D-mannitol, formic acid, L-malic acid, D-gluconic acid, N-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, mucic acid, myo-inositol, trehalose, inosine, maltose, gentiobiose, β-hydroxyl-D,L-butyric acid, cellobiose, glycyl-L-proline, L-pyroglutamic acid, *N*-acetyl-D-galacosamine, glycerol, L-fucose, α-keto-glutaric acid, melibiose, lactose, D-fucose, L-lactic acid, turanose, *N*-acetyl neuraminic acid, quinic acid, D-sorbitol, D-malic acid, p-hydroxy-phenylacetic acid, raffinose, γ-amino-butryric acid, L-arginine, stachyose, gelatin, D-serine, D-saccharic acid, methyl pyruvate, α-keto-butyric acid, bromo-succinic acid, L-serine, citric acid or acetic acid. Major fatty acids are $C_{16:1}$ ω5*c*, iso-$C_{15:0}$ and summed feature 3 ($C_{16:1}$ ω7*c* and/or iso-$C_{15:0}$ 2-OH). Polar lipids are phosphatidylethanolamine, three unidentified aminophospholipids and two unidentified polar lipids. The major respiratory quinone is MK7.

The type strain is 50A-KIRBA^T (=LMG 31080^T=CIP 111735^T), which was isolated from Kirchstaettbach, a small creek with medium conductivity and nearly neutral pH located in Obertrum, Austria. The genome of the type strain is characterized by a size of 3.1 Mbp and a G+C content of 38.4 mol%. The accession number of the genome sequence is JAANOP000000000 and for the 16S rRNA gene sequence it is MT140363.

DESCRIPTION OF *AQUIRUFA BEREGESIA* SP. NOV.

Aquirufa beregesia (be.re.ge'si.a. BRGS acronym of Bundesrealgymnasium Seekirchen; N.L. fem. adj. *beregesia*, from the BRG Seekirchen).

Cells form rods, about 1.5 µm long and 0.3 µm wide. Colonies grown on NSY or R2A agar are bright red, in older stages dark red, pigmented, circular and convex with smooth surface. Liquid cultures with NSY or R2A medium have an intense red-orange colouring. Cells are able to move on soft agar. Growth occurs at 5–30 °C and in 0–0.2% (w) NaCl. Cells assimilate pectin and Tween 40, weakly assimilate, acetoacetic acid, glucuronamide, D-fructose-6-PO_4 and acetic acid, and do not assimilate dextrin, sucrose, D-mannose, D-salicin, D-aspartic acid, L-histidine, propionic acid, D-lactic acid methyl ester, L-alanine, L-glutamic

acid, L-aspartic acid, D-glucose-6-PO_4, D-glucuronic acid, D-fructose, D-arabitol, α-D-glucose, α-hydroxy-butyric-acid, D-galactose, methyl β-D-glucoside, D-galacturonic acid, 3-methyl glucose, L-rhamnose, L-galactonic acid lactone, D-mannitol, formic acid, L-malic acid, D-gluconic acid, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, mucic acid, myo-inositol, trehalose, inosine, maltose, gentiobiose, β-hydroxyl-D,L-butyric acid, cellobiose, glycyl-L-proline, L-pyroglutamic acid, *N*-acetyl-D-galacosamine, glycerol, L-fucose, α-keto-glutaric acid, melibiose, lactose, D-fucose, L-lactic acid, turanose, *N*-acetyl neuraminic acid, quinic acid, D-sorbitol, D-malic acid, p-hydroxy-phenylacetic acid, raffinose, γ-amino-butryric acid, L-arginine, stachyose, gelatin, D-serine, D-saccharic acid, methyl pyruvate, α-keto-butyric acid, bromo-succinic acid, L-serine or citric acid. Major fatty acids are iso-$C_{15:0}$ and summed feature 3 ($C_{16:1}$ ω7*c* and/or iso-$C_{15:0}$ 2-OH). Polar lipids are phosphatidylethanolamine, one unidentified aminolipid, three unidentified aminophospholipids, one unidentified phospholipid and two unidentified polar lipids. The major respiratory quinone is MK7.

The type strain is 50C-KIRBA^T (=LMG 31501^T=CIP 111736^T), which was isolated from Kirchstaettbach, a small creek with medium conductivity and nearly neutral pH located in Obertrum, Austria. The genome of the type strain is characterized by a size of 3.2 Mbp and a G+C content of 38.5 mol%. The accession number of the genome sequence is SEWW00000000 and for the 16S rRNA gene sequence it is MK449346.

EMENDED DESCRIPTION OF *AQUIRUFA NIVALISILVAE* PITT *ET AL*. 2019

Aquirufa nivalisilvae [ni.va.li.sil'vae. L. adj. *nivalis*, snow covered; L. n. *silva*, forest; N.L. gen. n. *nivalisilvae*, from the forest of the village Schnee(snow)gattern].

Cells form rods, about 1.5 µm long and 0.5 µm wide. Colonies grown on NSY or R2A agar are bright red, in older stages dark red, pigmented, circular and convex with smooth surface. Liquid cultures with NSY or R2A medium have an intense red-orange colouring. Cells are able to glide on soft agar. Growth occurs at 5–36 °C and in 0–0.4% (w) NaCl. Cells assimilate Tween 40, weakly assimilate glucuronamide and sucrose, and do not assimilate L-histidine, propionic acid, D-lactic acid methyl ester, L-alanine, L-glutamic acid, L-aspartic acid, D-glucose-6-PO_4, D-glucuronic acid, D-fructose, α-hydroxy-butyric-acid, D-galactose, methyl β-D-glucoside, D-galacturonic acid, 3-methyl glucose, L-rhamnose, L-galactonic acid lactone, D-mannitol, formic acid, L-malic acid, D-gluconic acid, *N*-acetyl-β-D-mannosamine, mucic acid, myo-inositol, trehalose, inosine, maltose, gentiobiose, β-hydroxyl-D,L-butyric acid, cellobiose, glycyl-L-proline, L-pyroglutamic acid, glycerol, L-fucose, α-keto-glutaric acid, melibiose, lactose, D-fucose, L-lactic acid, turanose, *N*-acetyl neuraminic acid, quinic acid, D-sorbitol, D-malic acid, p-hydroxy-phenylacetic acid, raffinose, γ-amino-butryric acid, L-arginine, stachyose, gelatin, D-serine, D-saccharic acid, methyl pyruvate, α-keto-butyric acid, bromo-succinic acid, L-serine, citric acid and acetic acid. Major fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{15:0}$ 3-OH and summed feature 3 ($C_{16:1}$ ω6*c*, $C_{16:1}$ ω7*c* and iso-$C_{15:0}$ 2-OH). The only identified polar lipid is phosphatidylethanolamine. The major respiratory quinone is MK7.

The type strain is 59G-WUEMPEL^T (=LMG 31081^T=DSM 108554^T), which was isolated from a small intermittent freshwater pond with low conductivity and nearly neutral pH located near Lengau, Austria. The genome of the type strain is characterized by a size of 3.1 Mbp and a G+C content of 38.0 mol%. The accession number of the genome sequence is SEWX00000000 and for the 16S rRNA gene sequence it is MK449345.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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nov., reclassification of the order *Bdellovibrionales* in the class *Oligoflexia*, reclassification of the families *Bacteriovoracaceae* and *Halobacteriovoraceae* in the new order *Bacteriovoracales* ord. nov., and reclassification of the family *Pseudobacteriovoracaceae* in the order *Oligoflexales*. *Int J Syst Evol Microbiol* 2017;67:2555–2568.

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