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TAXONOMIC DESCRIPTION
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MICROBIOLOGY SOCIETY

Rariglobus hedericola gen. nov., sp. nov., belonging to the *Verrucomicrobia*, isolated from a temperate freshwater habitat

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Abstract

The bacterial strain 53C-WASEF was isolated from a small freshwater ditch located in Eugendorf, Austria. Phylogenetic reconstructions with 16S rRNA gene sequences and genome based, with amino acid sequences obtained from 105 single copy genes, suggested that the strain represents a new genus and a new species within the family *Opitutaceae*, which belongs to the class *Opitutae* of the phylum *Verrucomicrobia*. Comparisons of the 16S rRNA gene sequence of strain 53C-WASEF with those of related type strains revealed a highest sequence similarity of 93.5% to *Nibricoccus aquaticus* and of 92.9% to *Geminisphaera colitermitum*. Interestingly, phylogentic trees indicated the latter as being the closest known relative of the new strain. Phenotypic, chemotaxonomic and genomic traits were investigated. Cells were observed to be small, spherical, motile and unpigmented, and grew chemoorganotrophically and aerobically. The respiratory quinone was MK-7, the predominant fatty acids were anteiso-$C_{15:0}$, $C_{16:1}\omega5c$ and $C_{16:0}$. The identified polar lipids were phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. Genome sequencing revealed genes putatively encoding for flagella synthesis and cellulose degradation. The genome size was 4.1 Mbp and the G+C content 60.6 mol%. For the new genus and the new species, we propose the name *Rariglobus hedericola* gen. nov., sp. nov. (=CIP 111665^T=DSM 109123^T).

The division *Verrucomicrobia* was proposed 1997 by Hedlund *et al.* [1] based on the results of 16S rRNA gene sequence analysis of uncultured representatives from soil and aquatic habitats together with a few isolated strains. Currently, the taxon is ranked as a phylum [2]. Twenty years ago it was already known that the *Verrucomicrobia* are widespread in the environment and particularly abundant in soils. Despite that environmental significance only a few cultivated strains were available [3]. Recent studies with environmental samples revealed that their relative abundances in aquatic and terrestrial ecosystems are underestimated. This is likely due to a primer bias, as many of the commonly used PCR primers appear to exclude verrucomicrobial 16S rRNA genes during amplification [4]. With the availability of improved primers it was shown that the *Verrucomicrobia* might represent the most abundant phylum in particular temperate freshwater lakes [5]. Currently, only 21 genera and 51 species assigned to this phylum appear in the List of Prokaryotic Names with Standing in Nomenclature (LPSN) [6]. These low numbers of described taxa indicate that it is still challenging to cultivate members of the *Verrucomicrobia*. The family *Opitutaceae* belongs to the class *Opitutae* (subdivision 4) of the phylum and comprises seven validly published genera [7]. The genus *Lacunisphaera,* which is so far only effectively published, also belongs to this family. Overall, the family *Opitutaceae* contains 11 species. Remarkably, they originate from ecologically very distinct habitats, including an oil sand tailing pond [8], rice paddy soil [9], a lake [10], a hyporheic freshwater system [11], a hot spring [12], as well as guts of a termite [13–15], an ant [16] and a cockroach [17]. We isolated a new bacterial strain from a small temperate freshwater habitat. Strain 53C-WASEF^T was classified in the family *Opitutaceae* and obviously represents a new species of a new genus for which we propose the name *Rariglobus hedericola* gen. nov., sp. nov.

HOME HABITAT AND ISOLATION

Strain 53C-WASEF^T was isolated from a small water ditch in Eugendorf, Austria (47.861 N, 13.147 E). The water in this ditch flows for the most part below ground and reaches the

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Keywords: *Verrucomicrobia*; *Opitutaceae*; *Rariglobus*; freshwater.
Abbreviations: gAF, genome alignment fraction; gANI, whole genome average nucleotide identity; NSY, nutrient broth–soytone–yeast extract; R2A, Reasoner's 2A.
The Whole Genome Shotgun project and 16S rRNA gene sequence have been deposited at DDBJ/ENA/GenBank. The accession numbers of strain 53C-WASEF^T are VMBG00000000 and MN197844, respectively.
One supplementary table and two supplementary figures are available with the online version of this article.

Table 1. Traits characterizing strain 53C-WASEF^T

Fatty acids with amounts less than 0.5% are not listed. Concerning the assimilation patterns only positive results (except pectin) were shown, for the rest of the substrates of the GEN III MicroPlates (Biolog) negative results were obtained. +, Positive; –, negative; w, weak.

Characteristic	Strain 53C-WASEF^T
Cell morpology	Spherical
Mean diameter of cells (µm)	0.35
Pigmentation	Unpigmented
Temperature range for growth (°C)	6–30 (w)
Motility	+
NaCl tolerance (%)	0–0.7 (w)
Anaerobic growth on NSY plates	–
Anaerobic growth on NSY plates+nitrate	–
Catalase	–
Oxidase	–
Assimilation of:	
Acetoacetic acid	+
D-Fructose-6-PO_4	+
Glucuronamide	w
α-D-Glucose	w
Pectin	–
Fatty acids (%):	
$C_{12:0}$ 3-OH	1.4
iso-$C_{14:0}$	8.3
$C_{14:0}$	6.0
$C_{13:0}$ 2-OH	0.9
anteiso-$C_{15:0}$	31.1
iso-$C_{16:0}$	1.7
$C_{16:1}$ω11*c*	1.1
$C_{16:1}$ω5*c*	16.9
$C_{16:0}$	26.7
$C_{15:0}$ 2-OH	0.6
Iso-$C_{16:0}$ 3-OH	0.7
$C_{16:0}$ 3-OH	2.2
$C_{18:1}$ω5*c*	0.5

surface only for a short distance (Fig. S1, available in the online version of this article). Water was sampled in April 2018 as part of a Citizen Science project (Sparkling Science program), in which students from high schools cooperate with scientists [18]. The water sample was characterized by a pH of 6.8 and a conductivity of 978 µS cm^{-1}. The strain was isolated by filtering the water sample through a Minisart NML Plus (Sartorius) 0.65 µm pore size filter. The filtrate was subsequent plated on DTS (tryptone, soytone) agar plates [19], which contain, compared with R2A (Reasoner's 2A) medium [20], low nutrient concentrations. The agar plates were incubated at 20 °C in daylight. A selected colony was transferred to liquid R2A medium, three times alternate culturing in this medium and on R2A agar plates purified strain 53C-WASEF^T. The strain also grew in NSY (nutrient broth–soytone–yeast) medium [21].

PHENOTYPIC AND CHEMOTAXONOMIC CHARACTERIZATION

All phenotypic and chemotaxonomic characterizations were performed as described previously [18]. The maximum temperature of growth was tested on NSY agar plates at different temperatures in 1 °C steps starting at 26 °C; the incubation time was 2 weeks. Growth at 6 °C was also tested, but no temperatures below. NaCl tolerance was tested by using 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 NSY plates supplemented with 2 g l^{-1} $NaNO_3$ were used. Catalase activity was determined by bubble production in 3% (v/v) H_2O_2, and cytochrome oxidase activity was examined by using Bactident Oxidase test strips (Merck). Cell morphology and cell dimensions were determined by using DAPI (4′,6-diamidino-2-phenylindole) staining and an epifluorescence microscope (UV filter). Assimilation of various substrates was tested using GEN III MicroPlates (Biolog). The absorption was measured with a Multiskan FC (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 from 0.016 to 0.03 were regarded as weak utilization and >0.03 as positive. To test the cellulose-degrading ability of strain 53C-WASEF^T, cellulose Congo-red agar plates were used as described by Gupta *et al.* [22]. For testing motility, the strain was observed in a hanging drop. In addition, we used soft agar plates (1 g l^{-1} yeast extract, 0.1 g l^{-1} K_2HPO_4, 2.0 g l^{-1} agar) [23] coloured with 1.9 ml 0.5% phenol red to improve the visibility of the unpigmented cells. One drop of a well-growing culture was placed in the centre of these test plates, incubated at room temperature and observed for 3 weeks. The chemotaxonomic characterization of the strain included analyses of the composition of whole-cell fatty acids, polar lipids and respiratory quinones. These investigations 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 into liquid R2A medium and harvested after 3 days of growth by centrifuge (5000 x g). For the whole-cell fatty acid analysis an Agilent Technologies 6890 N instrument, the Microbial Identification System (MIDI) Sherlock version 6.1 and the TSBA 40 database were used as described by Sasser [24]. Polar lipids and respiratory quinones were extracted and analysed as described by Tindall [25, 26] based on the method by Bligh and Dyer [27].

Table 2. +Characteristics of genomes and gene content of strains 53C-WASEF^T and TAV2^T

Strains: 1, 53C-WASEF^T; 2, *Geminisphaera colitermitum* TAV2^T. +, Present; –, absent.

Characteristic	1	2
IMG/ER genome ID	2816332659	2517572100
Genome size (Mbp)	4.1	5.7
G+C content (mol%)	60.6	60.7
Genes putatively encoding for:		
Flagella synthesis (KEGG map ko02040)	+	+
Bacterial chemotaxis (KEGG pathway ko02030)	+	+
Type III secretion system (KEGG map ko03070)	+	+
Ass. nitrate reduction (EC:1.7.99.4)	+	–
Ass. nitrite reduction (EC:1.7.7.1)	+	–
Nitrogenase (EC:1.18.6.1)	–	+
Nitrous oxide reductase (EC:1.7.2.4)	–	+
Diss. nitrite reductase (EC:1.7.2.2)	–	+
Nitronate monooxygenase (EC:1.13.12.16)	–	+
Cellulose degradation:		
Endoglucanase (EC:3.2.1.4)	+	+
β-Glucosidase (EC:3.2.1.21)	+	+
Cellulase, glycosyl hydrolase family 5 (pfam00150)	+	+
Cytochrom c oxidase cbb3-type (KEGG module: M00156)	+	+
Fermentation product propionate and acetate (EC:2.7.2.1), lactate (EC:1.1.1.28)	–	+
Peroxidase (EC:1.11.1.9)	+	–
Pepidoglycan biosynthesis (KEGG map00550)	+	+
Fructose specific Pts system (KEGG map02060)	+	–
ABC-transporter (KEGG map02010):		
Nitrate/nitrite transport system	+	–
Alkanesulfonate transporter	–	+
Molybdate transporter	–	+
Iron III transporter	+	+
Branched-chain amino acid transporter	+	–
Iron II / manganase transporter	+	–
Na^+ transporter	+	–
Vitamin B12 transporter	–	+

Cells were small, spherical and unpigmented. They showed motility in liquid media as well as on soft agar plates. They grew very slowly in the used liquid media and on agar plates, respectively. On the latter they formed small, circular and convex colonies with smooth surfaces. From the substrates included in the GEN III MicroPlates, strain 53C-WASEF^T utilized only a small number (Table 1). Tests for cellulose degradation were not clear, perhaps due to the very small colonies of the strain on agar plates. The respiratory quinone was MK-7, the fatty acids are listed in Table 1 and the polar lipid pattern is shown in Fig. S2.

GENOMIC CHARACTERIZATION

DNA extraction and genome sequencing were performed as described previously [28]. 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 [29]. The obtained genome assembly consisted of 18 contigs including eight contigs with less than 1 Mbp. The k-mer coverage value was 116×, the N50 value was 2.4 Mbp and the L50 value was 1. The obtained genome sequence was annotated by using the NCBI Prokaryotic Genome Annotation Pipeline and deposited at GenBank. For further comparative analyses the genome was also annotated by using the Integrated Microbial Genomes and Microbiomes Expert Review (IMG/MER) annotation pipeline and incorporated in the IMG database [30]. The phylogenetic reconstructions (see below) revealed *Geminisphaera colitermitum* as closest relative of strain 53C-WASEF^T. Since the *G. colitermitum* type strain TAV2^T was mainly characterized by genomic traits [13–15], a gene content comparison between the two type strains was performed by using the IMG/MER system (Table 2). While strain 53C-WASEF^T possessed genes putatively encoding for assimilatory nitrate and nitrite reduction and uptake of nitrate/nitrite, strain TAV2^T lacked such genes. On the other hand, the latter taxon had genes putatively encoding for nitrogenases, nitrous oxide reductase, dissimilatory nitrite reductase and fermentation pathways, while these genes were not found in the genome of strain 53C-WASEF^T (Table 2). As reported for the effectively, but currently not validly published '*Lacunisphaera*' species, strains 53C-WASEF^T and TAV2^T possessed genes putatively encoding for peptidoglycan biosynthesis which appears to be unusual in the class *Opitutae* of the *Verrucomicrobia* [10]. For both strains, genes for complete glycolysis and citrate cycle were annotated in the IMG/MER database. Concerning the oxidative phosphorylation, genes putatively encoding for cytochrome c oxidase cbb3-type were found for both strains.

PHYLOGENY

Phylogenetic trees were calculated by using almost full-length sequences of the 16S rRNA gene (Fig. 1) and genome based, by using amino acid sequences of 105 single copy marker genes of 120 such genes recommended by Parks *et al.* [31] (Fig. 2). Both trees were reconstructed with members of the *Opitutaceae* and the *Puneicoccaceae*, the closest related family. For

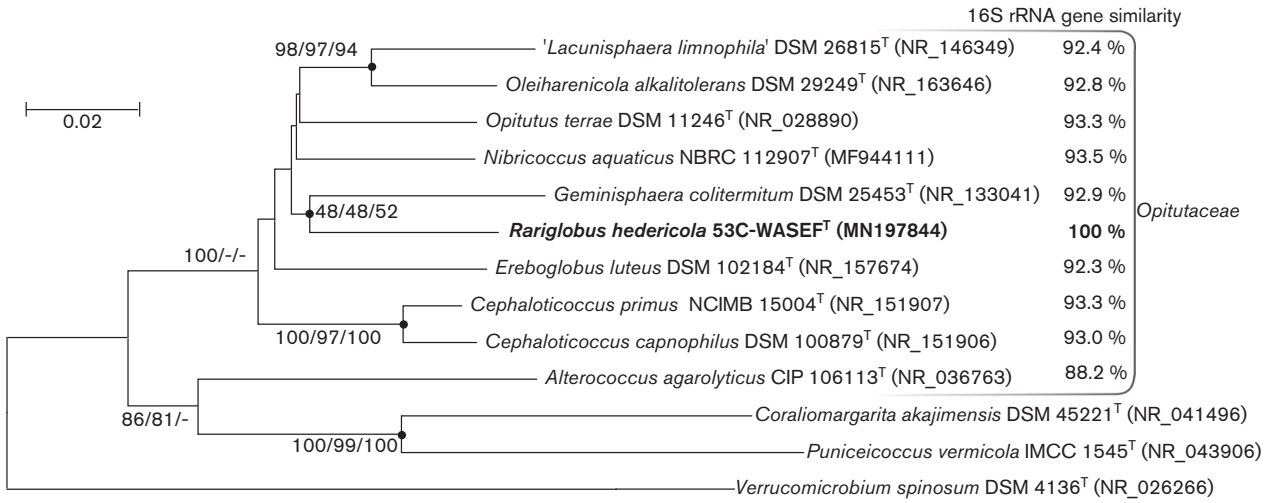

Fig. 1. Reconstruction of the phylogenetic position of the investigated strain based on almost full-length 16S rRNA gene sequences (1389 alignment positions). Shown is a neighbour-joining tree. Bootstrap values are shown from left to right for neighbour-joining, maximum-likelihood and maximum-parsimony trees calculated with the same sequence set. Bar, 0.02 substitutions per nucleotide position; dots indicate nodes present in all three calculated trees.

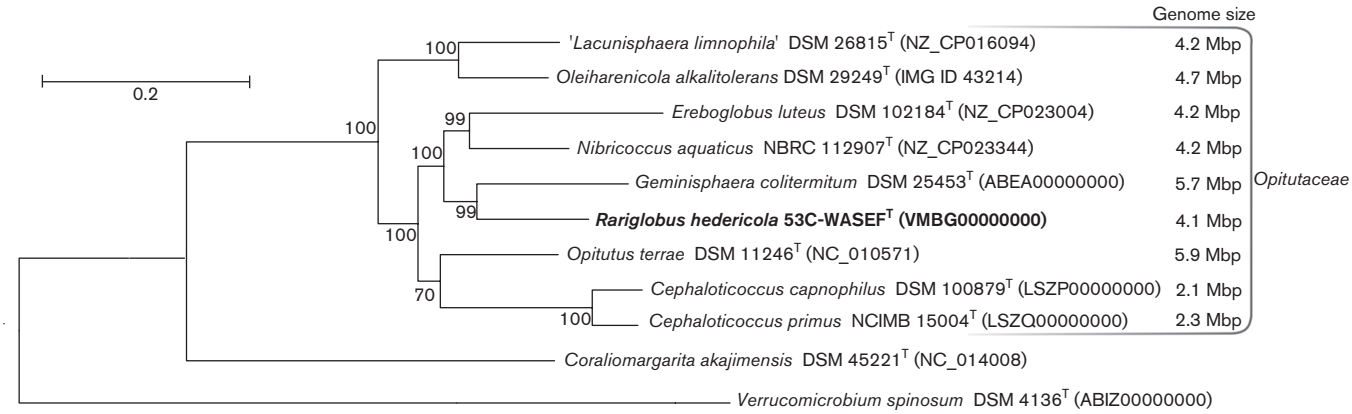

Fig. 2. Reconstruction of the phylogenetic position of the investigated strain based on amino acid sequences obtained from 105 single copy genes as a RAxML tree. Bar, 0.2 substitutions per nucleotide position.

the phylogenetic tree based on 16S rRNA genes (Fig. 1), the sequences were aligned, analysed for the best-fitting substitution model by using ModelTest and used for reconstruction of three phylogenetic trees with Kimura's two-parameter method [32], gamma distribution (five categories), invariant sites and 1000 bootstrap replications, by using the software MEGA X [33]. For the phylogenomic tree only 105 of the 120 protein-encoding single copy genes recommended by Parks *et al.* [31] were present in all investigated genomes. The 15 lacking genes are listed in Table S1. The amino acid sequences encoded by the genes were concatenated and aligned by using MAFFT [34]. The software GBLOCKS (version 0.91b) [35] was used to filter out highly variable positions, which resulted in a reduction of the alignment length from 45613 to 31286 positions in 455 selected blocks, which corresponded to 68% of the initial alignment positions. A RAxML tree [36] (Fig. 2) with 100 bootstrap replications was calculated by using the CIPRES Science Gateway version 3.3 [37].

The 16S rRNA gene tree placed strain 53C-WASEF^T within the family *Opitutaceae* on a branch together with *G. colitermitum*, but only with poor bootstrapping support. The amino acid sequence tree based on 105 marker genes confirmed this position and supported the common branch with *G. colitermitum* with a bootstrap value of 99%. This phylogenomic reconstruction gave better insights into the phylogeny of the family and revealed the nearest relatives of strain 53C-WASEF^T (Fig. 2).

ECOLOGY

It seemed that strain 53C-WASEF^T did not represent an abundant and widespread species. BLAST searches with its 16S

rRNA gene sequence revealed that available sequences from environmental samples showed only similarities of less than 98.7 %, which is the proposed cut-off for delineating different species [38]. Therefore, no hints on the occurrence of the new species in the environment were found. However, the above-mentioned primer bias could have resulted in overlooking of the new taxon in cultivation-independent diversity surveys of aquatic habitats. Alternatively, the new species may represent a habitat specialist, for example, specialised to the transition zone between aquatic and terrestrial habitats.

The gene content of the genome of strain 53C-WASEF^T hinted at a lifestyle of dwelling in transition zones characterized by steep environmental gradients. Evidence were genes putatively encoding for flagella synthesis and the observed motility of the strain. The presence of genes presumed for encoding assimilatory nitrate and nitrite reduction together with corresponding transport systems indicated the occurrence of strain 53C-WASEF^T in aerobic environments. Genes putatively encoding for peroxidase, which is predicted to be useful to avoid oxidative stress, also support this. Nevertheless, the presumed cytochrome c oxidase cbb3-type indicated the occurrence of the strain under microaerobic conditions [39]. From other members of the family *Opitutaceae*, a facultative anaerobic metabolism with fermentation pathways were reported [17]. Strain 53C-WASEF^T lacked genes putatively encoding propionate and acetate fermentation (Table 2), so it is likely that it occurs under aerobic or microaerobic conditions.

PROPOSAL OF *RARIGLOBUS HEDERICOLA* GEN. NOV., SP. NOV.

In both phylogenetic reconstructions with 16S rRNA gene sequences, as well as with 105 amino acid sequences of single copy genes, the new taxon was placed on a branch clearly separated from the other genera of the family *Opitutaceae* (Figs 1 and 2). In these trees, its closest relative was *G. colitermitum*. The branch length, i.e. evolutionary distance, of this taxon and strain 53C-WASEF^T was similar to those between other type strains belonging to different genera within the family *Opitutaceae*. 16S rRNA sequence similarity values of strain 53C-WASEF^T compared with type strains of other members of the family ranged between 88.2 (*Alterococcus agrolyticus*) and 93.5% (*Nibricoccus aquaticus*). Even the highest value is much lower than the cut-off of 94.5% proposed by Yarza *et al.* for separating two genera [40]. Pairwise calculated whole genome average nucleotide sequence identity (gANI) values are useful for prokaryotic species delineation, but they are not suitable for genus demarcation [41]. Correspondingly, in the case of *G. colitermitum*, the calculated gANI value with strain 53C-WASEF^T was 74.9 %, but the average genome alignment fraction (gAF) was only 17%. In the case of *N. aquaticus*, the gANI value was 75.0% and the gAF was 25.8 %. This means that the calculated gANI values were in the lower range of that common between species belonging to the same genus, but with unusual low gAF values.

Table 3. Comparison of characteristics of the new taxon and closely related species

Strain: 1, 53C-WASEF^T; 2, *Nibricoccus aquaticus*; 3, *Ereboglobus luteus*; 4, *Geminisphaera colitermitum*. ND, Not determined; –, negative; +, positive.

Species	1	2	3	4
Isolation source	Freshwater	Freshwater	Cockroach gut	Termite gut
Cell morphology	Cocci	Cocci	Cocci	Diplococci
Mean diameter of cells (μm)	0.35	0.45	0.6	0.55
Anaerobic growth	Obligate aerobic	Obligate aerobic	Facultatively anaerobic	Obligate aerobic*
Catalase	–	–	–	–
Oxidase	–	–	–	–
Pigmentation	Unpigmented	Yellow	Yellow	Unpigmented
Temperature range for growth (°C)	6–30	15–37	15–37	15–35
Motility	+	–	+	–
NaCl tolerance (%)	0–0.7	0–0.5	0–2.0	0–1.5
Predominant fatty acids:	$C_{14:0}$, $C_{16:0}$, iso-$C_{14:0}$, $C_{16:1}\omega5c$, anteiso-$C_{15:0}$	$C_{16:0}$, iso-$C_{14:0}$, iso-$C_{16:0}$, anteiso-$C_{15:0}$	ND	ND
G+C content (mol %)	60.6	62.2	59.7	60.5
Genome size (Mbp)	4.1	4.7	4.2	5.7
Reference(s)	This study	[11]	[17]	[13–15]

*Faculative anaerobic according to [17].

A comparison of strain 53C-WASEF^T and its closest relatives (Fig. 2) is given in Table 3. The type strain of the new genus is distinguishable by differences in some features from all its relatives. The temperature range of growth and tested motility differentiate strain 53C-WASEF^T from its closest relative *G. colitermitum*. In addition, the gene content, as well as the genome size differed between the two strains (Table 2). Furthermore, strain 53C-WASEF^T can be distinguished from its closest relatives by a combination of phenotypic and chemotaxonomic features (Table 3). Its ability to grow at temperatures between 6 and 30 °C and the small cell size is unique for the strain. Both phylogenetic reconstructions (Figs 1 and 2), as well as 16S rRNA gene similarities (Fig. 1), suggested that the strain represents a new genus and a new species within the family *Opitutaceae* for which we propose the name *Rariglobus hedericola* gen. nov., sp. nov.

DESCRIPTION OF *RARIGLOBUS* GEN. NOV.

Rariglobus gen. nov. (Ra.ri.glo'bus. L. masc. adj. *rarus*, rare; L. masc. n. *globus*, a sphere; N.L. masc. n. *Rariglobus*, a rare sphere-like bacterium).

Cells are coccoid and grow chemoorganotrophically and aerobically. The respiratory quinone is MK-7; predominant fatty acids are anteiso-$C_{15:0}$, $C_{16:1}$ω5*c* and $C_{16:0}$. Identified polar lipids are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. Based on phylogenetic reconstructions with 16S rRNA gene sequences and amino acid sequences obtained from 105 single-copy genes, respectively, the genus belongs to the family *Opitutaceae*. G+C content is approximately 60 mol% and genome size is about 4 Mbp. The type species of the genus is *Rariglobus hedericola*.

DESCRIPTION OF *RARIGLOBUS HEDERICOLA* SP. NOV.

Rariglobus hedericola sp. nov. [he.de.ri'co.la. L. fem. n. *Hedera*, a plant genus; L. suff. -*cola* (from L. n. *incola*), inhabitant; N.L. masc. n. *hedericola*, inhabitant of a *Hedera* plant].

Cells are coccoid, motile and approximately 0.35 µm in diameter. Catalase and oxidase results are negative. Cells grow chemoorganotrophically and aerobically. Cells grown on NSY agar form unpigmented, small and circular colonies. Growth occurs up to 30 °C and 0.7% (w) NaCl. Cells assimilate acetoacetic acid, D-fructose-6-PO_4, glucuronamide and α-D-glucose. Major fatty acids are iso-$C_{14:0}$, $C_{14:0}$, anteiso-$C_{15:0}$, $C_{16:1}$ω5*c* and $C_{16:0}$. The respiratory quinone is MK-7; polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, two unidentified phospholipids, an unidentified phosphoaminolipid and an unidentified lipid.

The type strain is 53C-WASEF^T (=CIP 111665^T=DSM 109123^T), which was isolated from a small water ditch located in Eugendorf (Austria). The G+C content of the genomic DNA of the type strain is 60.6mol% and the genome size is 4.1 Mbp.

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

References
1. Hedlund BP, Gosink JJ, Staley JT. *Verrucomicrobia* div. nov., a new division of the Bacteria containing three new species of *Prosthecobacter*. *Antonie van Leeuwenhoek* 1997;72:29–38.
2. Euzéby J. List of new names and new combinationspreviously effectively, but not validly, published. *Int J Syst Evol Microbiol* 2012;62:1–4.
3. Hugenholtz P, Goebel BM, Pace NR. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *J Bacteriol* 1998;180:4765–4774.
4. Bergmann GT, Bates ST, Eilers KG, Lauber CL, Caporaso JG *et al*. The under-recognized dominance of Verrucomicrobia in soil bacterial communities. *Soil Biology and Biochemistry* 2011;43:1450–1455.
5. Chiang E, Schmidt ML, Berry MA, Biddanda BA, Burtner A *et al*. *Verrucomicrobia* are prevalent in north-temperate freshwater lakes and display class-level preferences between lake habitats. *Plos One* 2018;13:20.
6. Parte AC. LPSN—list of prokaryotic names with standing in nomenclature. *Nucleic Acids Res* 2014;42:D613–D616.
7. Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures. Prokaryotic nomenclature up-to-date. August 2019..
8. Rochman FF, Kim J-J, Rijpstra WIC, Sinninghe Damsté JS, Schumann P *et al*. *Oleiharenicola alkalitolerans* gen. nov., sp. nov., a new member of the phylum *Verrucomicrobia* isolated from an oilsands tailings pond. *Int J Syst Evol Microbiol* 2018;68:1078–1084.
9. Chin KJ, Liesack W, Janssen PH. *Opitutus terrae* gen. nov., sp. nov., to accommodate novel strains of the division '*Verrucomicrobia*' isolated from rice paddy soil. *Int J Syst Evol Microbiol* 2001;51:1965–1968.
10. Rast P, Glöckner I, Boedeker C, Jeske O, Wiegand S *et al*. Three novel species with peptidoglycan cell walls form the new genus *Lacunisphaera* gen. nov. in the family *Opitutaceae* of the *Verrucomicrobial* subdivision 4. *Front Microbiol* 2017;8:18.
11. Baek K, Song J, Cho J-C, Chung EJ, Choi A. *Nibricoccus aquaticus* gen. nov., sp. nov., a new genus of the family *Opitutaceae* isolated from hyporheic freshwater. *Int J Syst Evol Microbiol* 2019;69:552–557.
12. Shieh WY, Jean WD. *Alterococcus agarolyticus*, gen.nov., sp.nov., a halophilic thermophilic bacterium capable of agar degradation. *Can J Microbiol* 1998;44:637–645.
13. Wertz JT, Kim E, Breznak JA, Schmidt TM, Rodrigues JLM. Genomic and physiological characterization of the *Verrucomicrobia* isolate *Diplosphaera colitermitum* gen. nov., sp. nov., reveals Microaerophily and nitrogen fixation genes. *Appl Environ Microbiol* 2012;78:1544–1555.
14. Wertz JT, Kim E, Breznak JA, Schmidt TM, Rodrigues JLM. Correction for Wertz *et al*., "Genomic and physiological characterization of the *Verrucomicrobia* isolate *Didymococcus colitermitum* gen. nov., sp. nov., reveals microaerophily and nitrogen fixation genes". *Appl Environ Microbiol* 2017;83:e00987–17.
15. Wertz JT, Kim E, Breznak JA, Schmidt TM, Rodrigues JLM. Second correction for Wertz *et al*., "Genomic and physiological

characterization of the *Verrucomicrobia* isolate *Geminisphaera colitermitum* gen. nov., sp. nov., reveals icroaerophily and nitrogen fixation genes". *Appl Environ Microbiol* 2018;84:e00952–18.

16. Lin JY, Russell JA, Sanders JG, Wertz JT. *Cephaloticoccus* gen. nov., a new genus of *'Verrucomicrobia'* containing two novel species isolated from *Cephalotes* ant guts. *Int J Syst Evol Microbiol* 2016;66:3034–3040.
17. Tegtmeier D, Belitz A, Radek R, Heimerl T, Brune A. *Ereboglobus luteus* gen. nov. sp. nov. from cockroach guts, and new insights into the oxygen relationship of the genera *Opitutus* and *Didymococcus (Verrucomicrobia: Opitutaceae*). *Syst Appl Microbiol* 2018;41:101–112.
18. Pitt A, Schmidt J, Koll U, Hahn MW. *Aquirufa antheringensis* gen. nov., sp. nov. and *Aquirufa nivalisilvae* sp. nov., representing a new genus of widespread freshwater bacteria. *Int J Syst Evol Microbiol* 2019;69:2739–2749.
19. Tamaki H, Tanaka Y, Matsuzawa H, Muramatsu M, Meng X-Y *et al*. *Armatimonas rosea* gen. nov., sp. nov., of a novel bacterial phylum, *Armatimonadetes* phyl. nov., formally called the candidate phylum OP10. *Int J Syst Evol Microbiol* 2011;61:1442–1447.
20. Reasoner DJ, Geldreich EE. A new medium for the enumeration and subculture of bacteria from potable water. *Appl Environ Microbiol* 1985;49:1–7.
21. Hahn MW, Stadler P, Wu QL, Pöckl M. The filtration–acclimatization method for isolation of an important fraction of the not readily cultivable bacteria. *J Microbiol Methods* 2004;57:379–390.
22. Gupta P, Samant K, Sahu A. Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. *Int J Microbiol* 2012;2012:5–.
23. Hahn MW, Schmidt J, Koll U, Rohde M, Verbarg S *et al*. *Silvanigrella aquatica* gen. nov., sp. nov., isolated from a freshwater lake, description of *Silvanigrellaceae* fam. nov. and *Silvanigrellales* ord. 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.
24. Sasser M. Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC Newsl* 1990;20:16.
25. Tindall BJ. A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* 1990;13:128–130.
26. Tindall BJ. Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* 1990;66:199–202.
27. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959;37:911–917.
28. Hoetzinger M, Schmidt J, Jezberová J, Koll U, Hahn MW. Microdiversification of a pelagic *Polynucleobacter* species is mainly driven by acquisition of genomic islands from a partially interspecific gene pool. *Appl Environ Microbiol* 2017;83:19.
29. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M *et al*. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455–477.
30. Chen I-MA, Chu K, Palaniappan K, Pillay M, Ratner A *et al*. IMG/M v.5.0: an integrated data management and comparative analysis system for microbial genomes and microbiomes. *Nucleic Acids Res* 2019;47:D666–D677.
31. Parks DH, Chuvochina M, Waite DW, Rinke C, Skarshewski A *et al*. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. *Nat Biotechnol* 2018;36:996–1004.
32. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
33. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 2018;35:1547–1549.
34. Katoh K, Kuma K, Toh H, Miyata T. MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res* 2005;33:511–518.
35. Castresana J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 2000;17:540–552.
36. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014;30:1312–1313.
37. Miller MA, Pfeiffer W, Schwartz T. Creating the CIPRES science gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*. New Orleans, LA: IEEE; 2010. pp. 1–8.
38. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR *et al*. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *Int J Syst Evol Microbiol* 2018;68:461–466.
39. Pitcher RS, Watmough NJ. The bacterial cytochrome cbb3 oxidases. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* 2004;1655:388–399.
40. Yarza P, Yilmaz P, Pruesse E, Glöckner FO, Ludwig W *et al*. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nat Rev Microbiol* 2014;12:635–645.
41. Qin Q-L, Xie B-B, Zhang X-Y, Chen X-L, Zhou B-C *et al*. A proposed genus boundary for the prokaryotes based on genomic insights. *J Bacteriol* 2014;196:2210–2215.