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Fluviispira multicolorata gen. nov., sp. nov. and *Silvanigrella paludirubra* sp. nov., isolated from freshwater habitats

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Abstract

Strain 33A1-SZDP^T was isolated from a small creek located in Puch, Austria. Strain SP-Ram-0.45-NSY-1^T was obtained from a small pond located in Schönramer Moor, Germany. 16S rRNA gene sequence similarities between the type strain of *Silvanigrella aquatica*, currently the only member of the family *Silvanigrellaceae*, and strains 33A1-SZDP^T and SP-Ram-0.45-NSY-1^T of 94.1 and 99.1%, respectively, suggested affiliation of the two strains with this family. Phylogenetic reconstructions with 16S rRNA gene sequences and phylogenomic analyses with amino acid sequences obtained from 103 single-copy genes suggested that the strains represent a new genus and a new species in the case of strain 33A1-SZDP^T (=JCM 32978^T=DSM 107810^T), and a new species within the genus *Silvanigrella* in the case of strain SP-Ram-0.45-NSY-1^T (=JCM 32975^T=DSM 107809^T). Cells of strain 33A1-SZDP^T were motile, pleomorphic, purple-pigmented on agar plates, putatively due to violacein, and showed variable pigmentation in liquid media. They grew chemoorganotrophically and aerobically and tolerated salt concentrations up to 1.2% NaCl (v/w). The genome size of strain 33A1-SZDP^T was 3.4 Mbp and the G+C content was 32.2 mol%. For this new genus and new species, we propose the name *Fluviispira multicolorata* gen. nov., sp. nov. Cells of strain SP-Ram-0.45-NSY-1^T were motile, pleomorphic, red-pigmented and grew chemoorganotrophically and aerobically. They tolerated salt concentrations up to 1.1% NaCl (v/w). The genome size of strain SP-Ram-0.45-NSY-1^T was 3.9 Mbp and the G+C content 29.3 mol%. For the new species within the genus *Silvanigrella* we propose the name *Silvanigrella paludirubra* sp. nov.

The family *Silvanigrellaceae* was described by Hahn *et al.* [1] as a novel family belonging to the novel order *Silvanigrellales*, assigned to the class *Oligoflexia*, phylum *Proteobacteria* [2]. Until now, the only validly described species within this family is *Silvanigrella aquatica*, with the type strain MWH-Nonnen-W8red^T, which was isolated from a small humic lake located in the Black Forest Mountains, Germany [1]. It is characterized by aerobic, chemoorganotrophic growing, pleomorphic, motile and red-pigmented cells, which tolerate salt concentrations up to 1.0% NaCl (w/v). Data are available for only one close relative of *S. aquatica*. This '*Candidatus* Spirobacillus cienkowskii', a pathogen of water fleas of the genus *Daphnia*, shares 96% of its 16S rRNA gene sequence with the type strain of *S. aquatica*. This taxon was morphologically and ecologically characterized almost 130 years ago [3], phylogenetically characterized in 2008 [4] and genome sequenced recently [5].

Within a Citizen Science project (Sparkling Science program), a cooperation between scientists and students from high schools, which pursues the aim to isolate and describe new bacterial taxa [6], water samples from various freshwater habitats were filtered and subsequently spread on agar plates. We found two isolates among the purified strains, which were affiliated with the family *Silvanigrellaceae*. Strain SP-Ram-0.45-NSY-1^T, which was red-pigmented, originated from a humic pond and obviously belonged to the genus *Silvanigrella*. Strain 33A1-SZDP^T, which originated from a small creek, showed a striking purple pigmentation and evidently represented a new genus of the family *Silvanigrellaceae*.

HOME HABITAT AND ISOLATION

The home habitat of strain 33A1-SZDP^T is a small creek, which flows through Puch as a tributary and discharges in the river Salzach, Austria. Water sampled in November 2017

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Keywords: *Silvanigrellaceae*; *Silvanigrella*; *Fluviispira*; freshwater.
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 for strain 33A1-SZDP^T are WFLN00000000 and MN574303, respectively. The accession numbers for strain SP-Ram-0.45-NSY-1^T WFLM00000000 and MN565732, respectively.
Two supplementary figures and two supplementary tables are available with the online version of this article.

at the approximate coordinates 47.7092 N and 13.0894 E, was characterized by a pH value of 6.6 and conductivity of 323 µS cm^{-1}. A second measurement at the same site in July 2018 revealed pH 8.2 and 332 µS cm^{-1}. The sample taken in 2017 was filtered through a 0.2 µm pore size filter and subsequently plated on Reasoner's 2A (R2A) agar plates [7]. The strain was purified by using nutrient broth–soytone–yeast extract (NSY) medium [8].

The home habitat of strain SP-Ram-0.45-NSY-1^T is a pond located at 47.8980 N and 12.8508 E in the Schönramer Moor in Bavaria, Germany, a disturbed peat bog. Water sampled in July 2017 was characterized by pH 6.7, conductivity of 28.6 µS cm^{-1}, temperature of 22.5 °C and oxygen saturation of 93.6% (7.7 mg l^{-1}). However, at other samplings of this habitat (n=16) pH values in the range pH 5.1–6.6 were obtained. The sampled water was filtered through a 0.45 µm pore size filter and subsequently spread on NSY agar plates [8] and purified by using this medium.

PHENOTYPIC AND CHEMOTAXONOMIC CHARACTERIZATION

All phenotypic and chemotaxonomic characterizations were performed as described previously [6] using NSY medium (pH 7.2) and an incubation temperature of 22 °C. In brief, the temperature range of growth was tested on NSY agar plates exposed at different temperatures in 1 °C steps, temperatures under 6 °C were not investigated. NaCl tolerance was tested by using NSY agar plates supplemented 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. Cell morphologies and cell dimensions were investigated 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 apparatus (Thermo Scientific) at a wavelength of 595 nm after 48 h incubation at 22 °C. After subtracting the value of the negative control (without substrate), obtained values below 0.015 were regarded as negative, values from 0.015 to 0.03 as weak utilization and >0.03 as positive. The chemotaxonomic characterizations of the strains 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 NSY medium (pH 7.2), incubated at 22 °C and harvested after 3 days of growth by centrifuge. For the whole-cell fatty acid investigations, 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 [9]. Polar lipids and respiratory quinones were extracted and analysed as described by Tindall [10, 11] based on the method of Bligh and Dyer [12]. For comparison, some of the investigations were performed with the type strain of *S. aquatica*, MWH-Nonnen-W8red^T. For temperature and salinity growth tests, as well as for the chemotaxonomic analysis, data were taken from previous investigations published elsewhere [1]. These investigations were performed in the same lab under the same conditions. One deviation was an incubation temperature of 28 °C for generation of biomass for the fatty acid investigations.

Phenotypic traits characterizing the three strains are given in Table 1. Strains 33A1-SZDP^T and SP-Ram-0.45-NSY-1^T showed variable morphologies (Fig. 1) similar to the morphologies of the type strain of *S. aquatica* (Fig. S1, available in the online version of this article). All strains occurred as rods of variable length and width, as well as filaments, straight, curved or spiral forms. Examples of the different morphologies are given in Fig. 1. Spirals and long filaments occurred in greater numbers when the strain was cultured on soft agar. Efforts to assign the various morphologies to different growth phases were unsuccessful, partly because the different forms co-occurred in samples taken at different times. Nevertheless, it seemed that cultures newly activated from glycerol stocks initially grew predominantly as short rods, while longer filaments and spirals appeared in greater numbers when the cultures were repeatedly sub-cultured. Strain SP-Ram-0.45-NSY-1^T was grey-red pigmented in liquid cultures and formed red colonies on agar plates (Table 1). Strain 33A1-SZPD^T showed various colours in liquid NSY media and purple colonies on agar plates (Table 1, Fig. 2), putatively due to synthesis of violacein (see below). For *S. aquatica* it was reported that experiments concerning the phenotypic characterization of the strain were also not reliable [1]. In respect to anaerobic growth, we also found that experiments gave variable results and were not replicable (Table 1). Concerning the assimilation patterns of substrates, the *S. aquatica* type strain used a broad variety of substrates, while strains SP-Ram-0.45-NSY-1^T and 33A1-SZDP^T assimilated a smaller number (Table 1). The results of the fatty acid analysis are given in Table 2. Strains 33A1-SZDP^T and SP-Ram-0.45-NSY-1^T contained menaquinone 8 and, as identified polar lipids, phosphatidylglycerol and phosphatidylethanolamine (Fig. S2). In strain SP-Ram-0.45-NSY-1^T, two unidentified lipids, two unidentified glycolipids, one unidentified aminolipid and one unidentified phospholipid were also detected, while strain 33A1-SZDP^T only had one additional unidentified lipid (Fig. S2) .

GENOMIC CHARACTERIZATION

DNA extraction and genome sequencing were performed as described previously [13]. 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 [14]. For strain SP-Ram-0.45-NSY-1^T, the *k*-mer coverage value was 43×, the *N50* value was 0.6 Mbp and the L50 value was 3. For strain 33A1-SZDP^T the *k*-mer coverage value was 95×, the *N50* value was 0.5 Mbp and the L50 value was also 3. The obtained genome sequences were annotated by using the NCBI Prokaryotic Genome Annotation Pipeline and deposited at GenBank. For further comparative

Table 1. Traits characterizing strains 33A1-SZDP^T, SP-Ram-0.45-NSY-1^T and MWH-Nonnen-W8red^T

Strains: 1, 33A1-SZDP^T; 2, SP-Ram-0.45-NSY-1^T; 3, *Silvanigrella aquatica* MWH-Nonnen-W8red^T. As regards the assimilation patterns, only results which were at least for one strain positive are shown, for the rest of the substrates of the GEN III MicroPlates (Biolog) all three strains were negative (see species description). +, Positive; –, negative; w, weak.

Characteristic	1	2	3
Cell morphology	Pleomorphic	Pleomorphic	Pleomorphic
Pigmentation, liquid media	Purple, grey, bright orange	Grey-red	Red
Pigmentation, agar plates	Purple	Red	Red
Motility on soft agar	+	+	+
Temperature range of growth (°C)	6–34 (w)	6–36 (w)	10–32 (w)
NaCl tolerance (%, v/w)	1.0–1.2 (w)	1.0–1.1 (w)	0–1.0 (w)
Anaerobic growth:			
NSY medium	–/w/+*	–/–*	–/–*
NSY medium with nitrate	–/–/+*	–/w*	–/w*
Assimilation of:			
Glucuronamide	–	+	+
Pectin	–	–	+
L-Histidine	+	+	+
Dextrin	–	–	+
α-D-Glucose	–	w	+
Tween 40	+	+	+
Mucic acid	–	–	+
Propionic acid	–	–	w
Formic acid	–	–	w
D-Glucose-6-PO_4	w	+	w
Acetic acid	–	–	w
D-Glucuronic acid	–	–	w
Gelatin	–	–	w
α-Keto-glutaric acid	–	+	w
D-Galacturonic acid	–	–	w
Acetoacetic acid	–	w	w
Quinic acid	–	–	w
D-Lactic acid methyl ester	+	–	w
α-Keto-butyric acid	–	–	w
D-Galactose	–	w	w
D-Mannose	–	–	w
L-Glutamic acid	–	+	w
L-Alanine	–	–	w
Lactose	–	–	w
D-Gluconic acid	w	–	–

Continued

Table 1. Continued

Characteristic	1	2	3
ß-Hydroxy-D,L-butyric acid	w	–	–
L-Malic acid	+	–	–
L-Arginine	w	–	–
Glycerol	w	–	–
D-Fructose	–	w	–
Melibiose	w	–	–
D-Fucose	–	+	–

*Results from different experiments.

analyses, the genomes were also annotated by the Integrated Microbial Genomes and Microbiomes Expert Review (IMG/MER) annotation pipeline and incorporated in the IMG database [15]. An overview of the newly sequenced genomes and of related genomes [strain MWH-Nonnen-W8red^T [1], strain RF1110005 (Shintani M and Kimbara K, unpublished) and '*Candidatus* Spirobacillus cienkowskii' binning01 [5]] is given in Table S1. Analyses with the IMG/MER system [15] revealed some interesting features. All five genomes contained genes putatively encoding for flagella compounds and assembly, as well as pilus assembly (Table S1), which confirmed the observed motility of our strains on soft agar plates (Table 1). In respect to the pigmentation, the five genomes included genes likely required for the biosynthesis of lycopene and ζ-carotene, especially crtl phytoene desaturases, putatively causal for the red colouring. Only strain 33A1-SZDP^T contained a gene putatively encoding for the biosynthesis of violacein, *VioE*, which matched the purple pigmentation of the strain. Only strains 33A1-SZDP^T and RF1110005 possessed genes that probably encoding for a formate dehydrogenase.

PHYLOGENY

Phylogenetic trees were calculated by using the almost full-length sequences of the 16S rRNA genes (Fig. 3) and by using the amino acid sequences of 103 single copy marker genes out of the 120 genes recommended by Parks *et al.* [16] (Fig. 4). The sequences of these marker genes were obtained from the genome sequences of the strains. The 17 genes, which are lacking in at least one of the analysed genomes are listed in Table S2. For the phylogenetic trees based on 16S rRNA genes (Fig. 3), the sequences were aligned, analysed for the best fitting substitution model and used for reconstruction of phylogenetic trees with the three methods maximum-likelihood, neighbour-joining and maximum-parsimony. The shown maximum-likelihood tree was calculated by

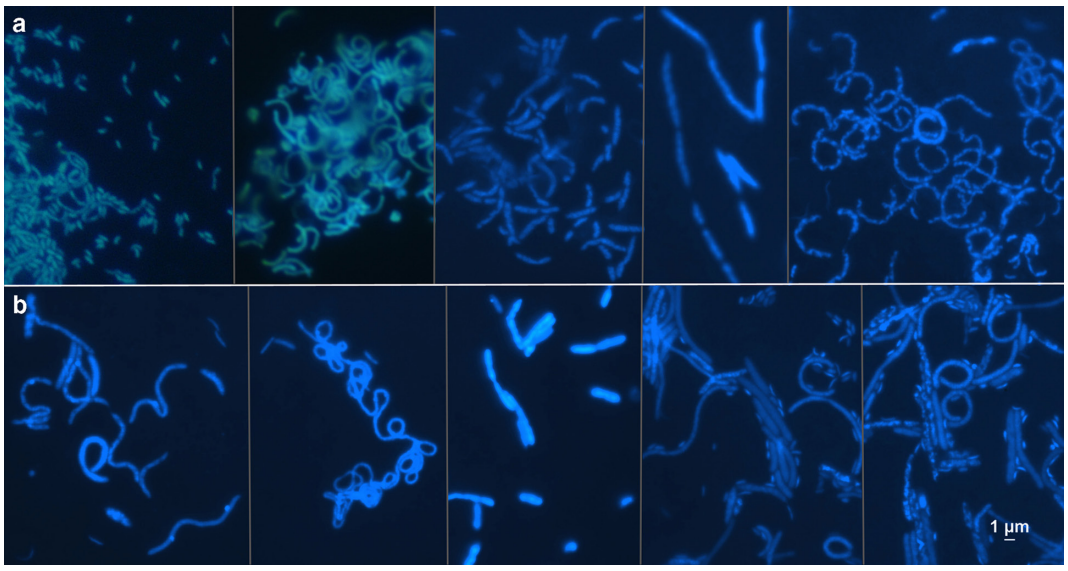

Fig. 1. Examples of the different morphologies of strains 33A1-SZPD^T (a, first row) and SP-Ram-0.45-NSY-1T (b, second row). Cells from various cultures were stained with DAPI and observed under an epifluorescence microscope (UV filter). Bar, 1µm.

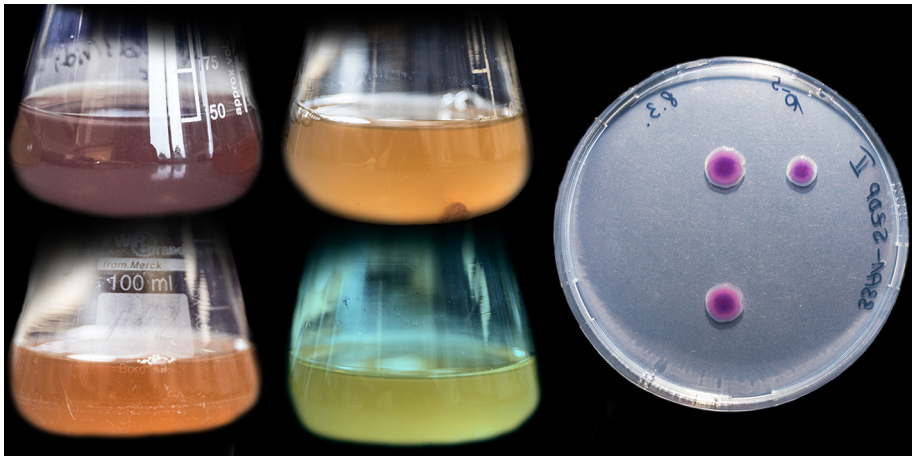

Fig. 2. Pigmentation of strain 33A1-SZDP^T grown in liquid NSY medium (left) and on an NSY agar plate (right).

using the Kimura two-parameter substitution model [17], gamma distribution (five categories), invariant sites, and 1000 bootstrap replications, by using the software MEGAX [18]. All positions of the 1338 aligned positions with less than 95% site coverage were eliminated, which resulted in 1226 used positions. For the phylogenomic tree the amino acid sequences of the used 103 protein-encoding single-copy genes were concatenated and aligned by using MAFFT [19]. The software GBLOCKS (version 0.91b) [20] was used to filter out highly variable positions, which resulted in a reduction of the alignment length from 47886 to 27328 positions in 452 selected blocks, which corresponded to 57% of the initial alignment positions. A RAxML tree [21] (Fig. 4) with 100 bootstrap replications was calculated using the CIPRES Science Gateway version 3.3 [22].

Both phylogenetic trees showed the same structure, nevertheless, the two earliest nodes in the 16S rRNA gene tree (Fig. 3) were only present in the tree established by using the maximum-likelihood algorithm. All other bootstrap values were very high; in the genome-based phylogenetic reconstruction (Fig. 4) all internal nodes were supported by bootstrap values of 100%. The phylogenetic reconstructions assigned both new strains to the family *Silvanigrellaceae*, placed strain SP-Ram-0.45-NSY-1^T on a branch beside *S. aquatica* and separated strain 33A1-SZDP^T from the *Silvanigrella*/Spirobacillus group.

ECOLOGY

As reported previously [1], the currently known members of the family *Silvanigrellaceae* seem to be rather rare species. For strain 33A1-SZPD^T, BLAST searches with the 16S rRNA gene sequence, considering the cut off for new species of 98.7% [23], revealed neither sequences from environmental samples nor from cultures that could be assigned to the proposed novel species represented by the strain. Regarding the novel proposed genus, only one hit was found. Strain RF1110005, which seems to belong to the same genus as strain 33A1-SZDP^T (Figs 3 and 4), was isolated from brackish Lake Sanaru in Hamamatsu, Japan (Shintani M and Kimbara K, unpublished). Interestingly, this eutrophic brackish lake [24] shares with the home habitat of 33A1-SZPD^T in comparison to the habitats of the *Silvanigrella* strains a much higher conductivity (>10 fold) and an alkaline pH. In the case of strain SP-Ram-0.45-NSY-1^T, five bacterial strains isolated from amphibian skin [25] shared 99.8% 16S rRNA gene sequence identity and likely belonged to the same species. Taking the mean cut-off of 94.6% separating two genera [26] into account, three uncultured clones which seemed to belong to the genus *Silvanigrella* were found: from Yellostone Lake, USA (accession number HM856518) [27], from a peat bog habitat in Tierra del Fuego, Argentina (JF907335) (Kip N *et al.*, unpublished) and from a subsurface karst water pool in Switzerland (HE998901) [28]. The physical–chemical conditions of the home habitats of strains SP-Ram-0.45-NSY-1^T and MWH-Nonnen-W8red^T had the occurrence of humic matter, low conductivity and pH slightly below neutral in common. One could speculate that the genus *Silvanigrella* occurs more frequently under humic and low conductivity conditions and members of the genus *Fluviispira* more frequently in systems with higher conductivity and pH above neutral; however, more data are needed to support these postulated trends.

An interesting feature is the violacein synthesis predicted for strain 33A1-SZDP^T. This pigment is known for antibacterial activity and potential antitumour effects, and was shown to act against some diseases caused by eukaryotic parasites [29]. A study suggested that presence of violacein-producing bacteria on frog skin prevented morbidity and mortality caused by a lethal skin fungus [30]. The ecological role of violacein in strain 33A1-SZDP^T is currently unknown, and it is also not known how widespread genes for synthesis of this pigment are among *Silvanigrellales* bacteria.

Another interesting point related to the ecology of the strains investigated so far is the morphological variability. They share this trait with the parasitic '*Candidatus*

Table 2. Composition of the fatty acids of strains SP-Ram-0.45-NSY-1^T, 33A1-SZDP^T and MWH-Nonnen-W8red^T

Strains: 1, 33A1-SZDP^T; 2, SP-Ram-0.45-NSY-1^T; 3, *Silvanigrella aquatica* MWH-Nonnen-W8red^T. Only fatty acids with results of higher than 0.5% in at least one of the strains are listed. Data for strain 3 were published elsewhere [1].

Fatty acid	1	2	3
iso-$C_{15:0}$	20.8	33.1	33.1
$C_{17:1}\omega8c$	12.0	8.1	9.1
iso-$C_{14:0}$	11.1	5.9	1.3
iso-$C_{16:0}$	9.8	1.2	1.1
anteiso-$C_{15:0}$	9.3	5.7	11.2
$C_{15:0}$	6.6	5.8	3.6
$C_{17:0}$	5.8	2.9	6.5
Summed feature 3*	4.5	5.0	5.7
$C_{15:1}\omega6c$	3.1	3.8	2.7
iso-$C_{13:0}$–3OH	2.8	0.1	–
iso-$C_{14:0}$–3OH	2.3	10.6	5.0
$C_{16:0}$	2.3	1.8	4.3
$C_{17:0}$–3OH	1.9	0.6	0.6
iso-$C_{17:0}$	1.1	1.6	5.6
$C_{14:0}$	0.9	0.8	1.0
Unknown 13.565*	0.5	5.4	1.4
iso-$C_{17:0}$–3OH	0.5	0.5	1.3
anteiso-$C_{17:0}$	0.5	0.3	0.8
Summed feature 2*	0.4	2.6	0.4
iso-$C_{15:0}$–3OH	–	–	1.2
iso-$C_{11:0}$–3OH	–	0.2	0.7

*Unknown 13.565, unknown fatty acid with equivalent chain length of 13.565; summed feature 2, iso-$C_{16:0}$ I, $C_{14:0}$–3OH; summed feature 3, $C_{16:1}\omega6c$, $C_{16:1}\omega7c$, iso-$C_{15:1}$–2OH, iso-$C_{15:0}$–2OH.

Spirobacillus cienkoskii'. However, it remains unclear if the observed variations in morphologies of the non-parasitic strains are an artificial effect of the cultivation conditions or if they occur as well under natural conditions. If so, the ecological significance could be that the diverse forms allow these bacteria species to adapt to variable environmental conditions. Such morphological stages could represent resting stages, migratory stages, or in the case of larger filaments or large spirilla, stages less sensitive to protistan predation (morphological defence strategies). However, more investigations are needed to prove the links between growth conditions, for example substrate availability, and morphological stages of the *Silvanigrellaceae* strains.

PROPOSAL OF *SILVANIGRELLA PALUDIRUBRA* SP. NOV.

Both phylogenetic reconstructions with 16S rRNA gene sequences and with 103 amino acid sequences of single-copy genes, placed strain SP-Ram-0.45-NSY-1^T in the family *Silvanigrellaceae* on a branch beside *S. aquatica*. To test if the new strain represents a new species pairwise whole genome average nucleotide identity (gANI) values were calculated with the IMG/MER system [15] (Table S1). Although the 16S rRNA gene sequence similarity between the two taxa is relatively high, i.e. 99.1% (Table S1), the pairwise calculated gANI value of 75.6% indicated clearly that the new strain does not belong to the species *S. aquatica* [31–34]. Furthermore, several phenotypic and chemotaxonomic features distinguished the two taxa. While strain SP-Ram-0.45-NSY-1^T was grey-red pigmented in liquid cultures, utilized only a small number of substrates (Table 1) and grew up to 36 °C, strain MWH-Nonnen-W8red^T (*S. aquatica*) showed an intense red colouring, utilized a much broader variety of substrates and grew only up to a temperature of 32 °C. Even though the biomass for the fatty acids was grown at different temperatures, the fatty acid pattern of the two taxa were nearly the same but differed slightly in the amount (see Table 2). While MK8 was identified as the menaquinone detected in strain SP-Ram-0.45-NSY-1^T no known quinone could be identified for strain MWH-Nonnen-W8red^T. So, we assume that strain SP-Ram-0.45-NSY-1^T represents a new species of the genus *Silvanigrella*, for which we propose the name *Silvanigrella paludirubra* sp. nov.

PROPOSAL OF *FLUVIISPIRA MULTICOLORATA* GEN. NOV., SP. NOV.

Both phylogenetic reconstructions with 16S rRNA gene sequences and with 103 amino acid sequences of single-copy genes placed strain 33A1-SZDP^T in the family *Silvanigrellaceae* on a separated branch but close to *S. aquatica*. The 16S rRNA sequence similarity between the latter and 33A1-SZDP^T was 94.3% and therefore slightly lower than the threshold sequence identity of 94.5% proposed by Yarza *et al.* [26] for separation of two genera. Some phenotypic and chemotaxonomic features distinguished strain 33A1-SZDP^T from the two strains belonging to the genus *Silvanigrella*. While the latter were basically red pigmented, showed the new strain various pigmentations in liquid medium and a purple colour on agar plates (Table 3). Furthermore, the assimilation patterns of substrates differed between the two taxa (Table 3). So, we assume that strain 33A1-SZDP^T represents a new genus and a new species, for which we propose the name *Fluviispira multicolorata* gen. nov, sp. nov.

DESCRIPTION OF *SILVANIGRELLA PALUDIRUBRA* SP. NOV.

Silvanigrella paludirubra sp. nov. (pa.lu.di.ru'bra L. fem. n. *palus*, bog; L. fem. adj. *rubra*, red; N.L. fem. n. *paludirubra*, the red from the bog).

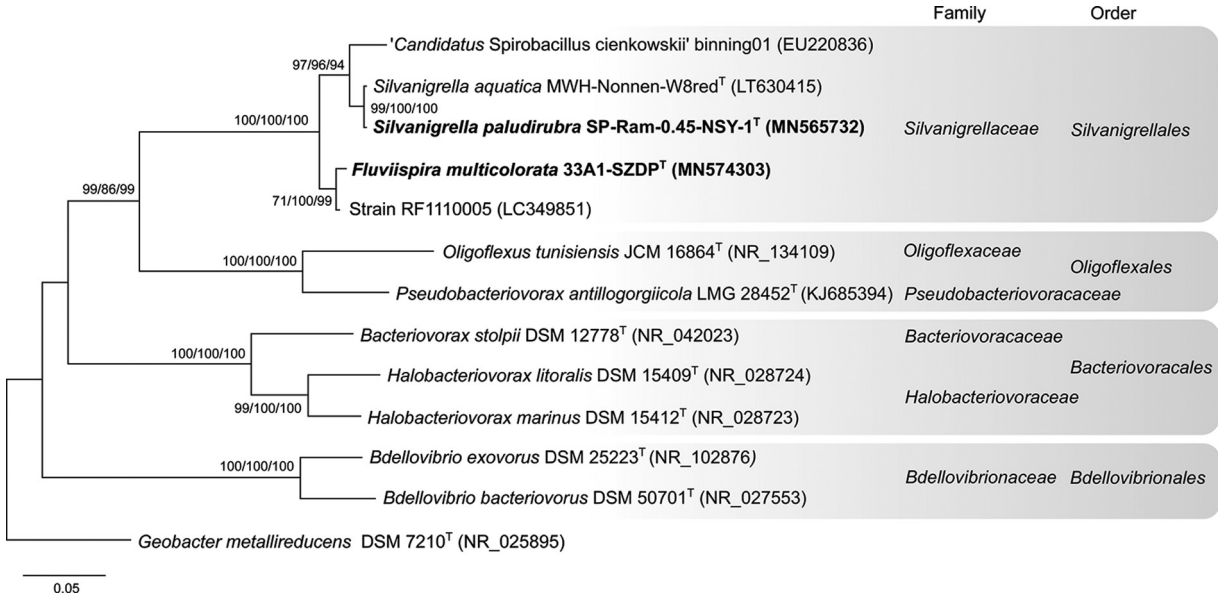

Fig. 3. Reconstruction of the phylogenetic position of the investigated strains within the class *Oligoflexia* based on almost full-length 16S rRNA gene sequences. Shown is a maximum-likelihood tree. Bootstrap values are shown from left to right for maximum-likelihood, neighbour-joining and maximum-parsimony trees calculated with the same sequence set. Bar, 0.05 substitutions per nucleotide position.

Cells are motile and pleomorphic, including rods and filaments of various length and formation. They grow chemoorganotrophically and aerobically. Cells grown on NSY agar form red-pigmented, small and circular colonies. In liquid NSY medium they appear grey-red-pigmented. Growth occurs up to 36 °C and 1.1% NaCl (v/w). Cells assimilate glucuronamide, L-histidine, α-D-glucose, Tween 40, D-glucose-6-PO_4, α-keto-glutaric acid, acetoacetic acid, D-galactose, L-glutamic acid, D-fructose and D-fucose; do not assimilate *N*-acetyl-D-glucosamine, 3-methyl glucose, lactose, glycerol, L-fucose, melibiose, maltose, acetic acid, raffinose, L-aspartic acid, glycyl-L-proline, D-sorbitol, dextrin, cellobiose, D-lactic acid methyl ester, D-glucuronic acid, turanose, inosine, gentiobiose, *N*-acetyl-D-galactosamine, D-mannose, myo-inositol, D-serine, trehalose, L-alanine, α-keto-butyric acid, γ-amino-butyric acid, D-arabitol, gelatin, L-rhamnose, D-gluconic acid, stachyose, D-fructose-6 -PO_4, L-galactonic acid lactone, methyl β-D-glucoside, propionic acid, D-galacturonic acid, L-arginine, sucrose, L-lactic acid, D-aspartic acid, β-hydroxy-D,L-butyric acid, quinic acid, L-malic acid, D-mannitol, pectin, *N*-acetyl-β-D-mannosamine, D-salicin, L-serine, mucic acid, *N*-acetyl neuraminic acid, L-pyroglutamic acid, D-saccharic

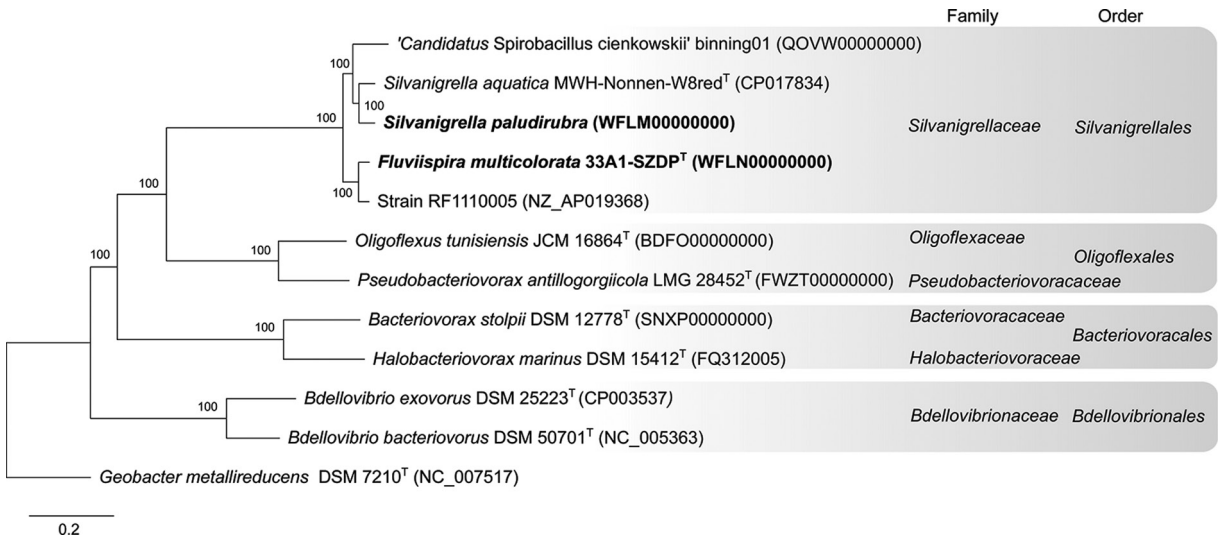

Fig. 4. Reconstruction of the phylogenetic position of the investigated strains within the class *Oligoflexia* based on amino acid sequences obtained from 103 single-copy genes as a RAxML tree. Bar, 0.2 substitutions per nucleotide position.

Table 3. Features differentiating the genus *Fluviispira* from the genus *Silvanigrella*

w, Weak; +, positive; –, negative.

Characteristic	*Fluviispira*	*Silvanigrella*
Pigmentation, liquid media	Purple, grey, bright orange	Grey-red
Pigmentation, agar plates	Purple	Red
Assimilation of:		
Glucuronamide	–	+
α-D-Glucose	–	+
α-Keto-glutaric acid	–	+
Acetoacetic acid	–	w
D-Galactose	–	w
L-Glutamic acid	–	+
D-Gluconic acid	w	–
ß-Hydroxy-D,L-butyric acid	w	–
L-Malic acid	+	–
L-Arginine	w	–
Glycerol	w	–
Melibiose	w	–

acid, α-hydroxy-butyric acid, citric acid, formic acid, bromo-succinic acid, methyl pyruvate, p-hydroxy-phenylacetic acid and D-malic acid. Major fatty acids (more than 5%) are iso-$C_{15:0}$, $C_{17:1}\omega8c$, iso-$C_{14:0}$, anteiso-$C_{15:0}$, $C_{15:0}$, iso-$C_{14:0}$–3OH and an unknown component with an equivalent chain length of 13.565. The respiratory quinone is MK-8. The polar lipids are phosphatidylglycerol, phosphatidylethanolamine, one unidentified phospholipid, two unidentified glycolipids, two unidentified lipids and one aminolipid.

The type strain is SP-Ram-0.45-NSY-1^T (=JCM 32975^T=DSM 107809^T), which was isolated from a small pond located in Schönramer Moor, Bavaria, Germany. The G+C content of the genomic DNA of the type strain is 29.3% and the genome size is 3.9 Mbp.

DESCRIPTION OF *FLUVIISPIRA* GEN. NOV.

Fluviispira gen. nov. (Flu.vi.i.spi′ra. L. masc. n. *fluvius*, river; L. fem. n. *spira*, coil; N.L. fem. n. *Fluviispira*, a coil from a river).

Cells are motile and pleomorphic, including rods and filaments of various length and formation. They grow chemoorganotrophically and aerobically and occur in various pigmentations. The respiratory quinone is MK-8. The polar lipids are phosphatidylglycerol and phosphatidylethanolamine. The major fatty acid is iso-$C_{15:0}$. Based on phylogenetic reconstructions with 16S rRNA gene sequences and amino acid sequences obtained from 103 single-copy genes, respectively, the genus belongs to the family *Silvanigrellaceae*. The G+C content of the genomic DNA is approximately 33 mol% and the genome size 3.5 Mbp. The type species of the genus is *Fluviispira multicolorata*.

DESCRIPTION OF *FLUVIISPIRA MULTICOLORATA* SP. NOV.

F. multicolorata sp. nov. (mul.ti.co.lo.ra′ta. L. masc. adj. *multus*, many; L. past part. *coloratus*, coloured; N.L. fem. adj. *multicolorata*, multi-coloured).

Cells are motile and pleomorphic, including rods and filaments of various length and formation. They grow chemoorganotrophically and aerobically. Cells grown on NSY agar form purple-pigmented, circular colonies. Liquid cultures appear either purple, grey or orange. Growth occurs up to 34 °C and 1.2% (v/w) NaCl. Cells assimilate L-histidine, Tween 40, D-glucose-6-PO_4, D-lactic acid methyl ester, D-gluconic acid, β-hydroxy-D,L-butyric acid, L-malic acid, L-arginine, glycerol and melibiose; do not assimilate D-fructose, D-fucose, glucuronamide, α-D-glucose, *N*-acetyl-D-glucosamine, α-keto-glutaric acid, acetoacetic acid, 3-methyl glucose, lactose, D-galactose, L-glutamic acid, L-fucose, maltose, acetic acid, raffinose, L-aspartic acid, glycyl-L-proline, D-sorbitol, dextrin, cellobiose, D-glucuronic acid, turanose, inosine, gentiobiose, *N*-acetyl-D-galactosamine, D-mannose, myo-inositol, D-serine, trehalose, L-alanine, α-keto-butyric acid, γ-amino-butryric acid, D-arabitol, gelatin, L-rhamnose, stachyose, D-fructose-6-PO_4, L-galactonic acid lactone, methyl β-D-glucoside, propionic acid, D-galacturonic acid, sucrose, L-lactic acid, D-aspartic acid, quinic acid, D-mannitol, pectin, *N*-acetyl-β-D-mannosamine, D-salicin, L-serine, mucic acid, *N*-acetyl neuraminic acid, L-pyroglutamic acid, D-saccharic acid, α-hydroxy-butyric acid, citric acid, formic acid, bromo-succinic acid, methyl pyruvate, p-hydroxy-phenylacetic acid and D-malic acid. Major fatty acids (more than 5 %) are iso-$C_{15:0}$, $C_{17:1}\omega8c$, iso-$C_{14:0}$, iso-$C_{16:0}$, anteiso-$C_{15:0}$, $C_{15:0}$ and $C_{17:0}$. The respiratory quinone is MK-8. The polar lipids are phosphatidylglycerol, phosphatidylethanolamine and one unidentified lipid.

The type strain is 33A1-SZDP^T (=JCM 32978^T=DSM 107810^T), which was isolated from a small creek flowing through Puch as an inflow to the river Salzach, Austria. The G+C content of the genomic DNA of the type strain is 32.2 mol% and the genome size is 3.4 Mbp.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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