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 Halobacteriovoracaceae in the new order Bacteriovoracales ord. nov., and reclassification of the family Pseudobacteriovoracaceae in the order Oligoflexales

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Abstract

The unusual chemo-organoheterotrophic proteobacterial strain MWH-Nonnen-W8red1 was isolated from a lake located in the Black Forest (Schwarzwald), Germany, by using the filtration-acclimatization method. Phylogenetic analyses based on the 16S rRNA gene sequence of the strain could not provide clear hints on classification of the strain in one of the current classes of the phylum Proteobacteria. Whole-genome sequencing resulted in a genome size of 3.5 Mbp and revealed a quite low DNA G+C content of 32.6 mol%. In-depth phylogenetic analyses based on alignments of 74 protein sequences of a phylogenetically broad range of taxa suggested assignment of the strain to a new order of the class Oligoflexia. These analyses also suggested that the order Bdellovibrionales should be transferred from the class Deltaproteobacteria to the class Oligoflexia, that this order should be split into two orders, and that the family Pseudobacteriovoracaceae should be transferred from the order Bdellovibrionales to the order Oligoflexales. We propose to establish for strain MWH-Nonnen-W8red1 (=DSM 23856=CCUG 58639) the novel species and genus Silvanigrella aquatica gen. nov., sp. nov. to be placed in the new family Silvanigrellaceae fam. nov. of the new order Silvanigrellales ord. nov.

Strain MWH-Nonnen-W8red1 was isolated from a freshwater lake located in the Black Forest Mountains (Schwarzwald), Germany. Analyses of the strain’s 16S rRNA gene sequence indicated that this strain is only distantly related to any type strain described previously. BLAST searches against sequences of type material revealed that the top hits (November 2016) represent type strains belonging to various classes of the phylum Proteobacteria. The best hit, Vulgatibacter incomptus DSM 277101 (class Deltaproteobacteria), shared a 16S rRNA gene sequence similarity of 85%, while various type strains affiliated with the classes Gammaproteobacteria and Acidithiobacillia shared similarities of 81–82%. Inclusion of non-type-material in BLAST searches resulted in much higher sequence similarity values. Interestingly, the uncultured taxon Spirobacillus cienkowskii, a pathogen of water flea (Daphnia spp.), which was described by Élie Metchnikoff almost 130 years ago [1] and subsequently rediscovered a few years ago by Rodrigues and colleagues [2], shared a 16S rRNA gene sequence similarity of 96%. Other taxonomically unclassified cultured and uncultured bacteria even shared 97–99% 16S rRNA gene sequence similarities [3–6]. According to Nakai and colleagues, who recently described the new class

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Keywords: Oligoflexia; Bdellovibrionales; genome; reclassification; new order.

Abbreviations: AA, average amino acid identity; ANI, average nucleotide identity; IMG, Integrated Microbial Genomics; OD, optical density.

The GenBank/EMBL/DDBJ accession numbers for the whole-genome sequence of Silvanigrella aquatica MWH-Nonnen-W8red1 are CP017834–CP017838.

Seven supplementary figures and five supplementary tables are available with the online Supplementary Material.
Oligoflexia of the phylum Proteobacteria, ‘Spirobacillus ciankowskii’ and related strains may represent a novel class of the phylum Proteobacteria [7].

We characterized strain MWH-Nonnen-W8redT by following the polyphasic approach and included genome sequencing and comparative analysis of the annotated genome sequence of the strain. Based on the results obtained, we propose that this strain represents a novel species, genus, family and order affiliated with the class Oligoflexia Nakai et al. 2014 [7] within the phylum Proteobacteria.

Strain MWH-Nonnen-W8redT was obtained from Lake Nonnenmattweiher located (geographic coordinates 47.795299° N and 7.798552° E) in the Black Forest Mountains (Schwarzwald), Germany, at a height above sea level of 926 m. The lake has a surface area of 71 ha and is characterized by a floating peat moss island. The lake is located at the site of a former glacial cirque lake, which was naturally infilled and replaced by a mire in the Middle Ages. The current lake was established by construction of an embankment dam in 1722, lost its water for a couple of years due to dam failure in 1922 and was re-established in the early 1930s. The current lake can be characterized as a shallow softwater lake influenced by a mire. Surface waters (about 10–20 cm depths) of the lake were sampled from the shore line by using a water sampling dipper. At the day of sampling (27 July 2008), the water temperature was 19.4 °C, the pH was 6.7 and conductivity was 21.8 µS cm⁻¹. The water was slightly stained by dissolved humic matter (absorption of 0.2µm-filtered water at a wavelength of 250 nm of 0.12).

Strain MWH-Nonnen-W8redT was isolated by using the filtration-acclimatization method [8], which included filtration of a water sample through a filter with a pore size of 0.2 µm and stepwise acclimatization to higher substrate concentrations. Liquid and solidified (1.5 % agar) NSY medium [8], which mainly consists of equal amounts of nutrient broth, soytone and yeast extract (all three from Difco, BD International) was used for isolation and maintenance of the strain. The isolate was stored at −70°C in NSY medium plus 15 % (w/v) glycerol prior to deposition of the strain in public culture collections.

Strain MWH-Nonnen-W8redT could be grown on NSY or R2A medium [9]. Comparative tests with R2A medium of different strength suggested that dilution of the medium to half the standard concentration accelerated growth (turbidity after 2 days). The strain formed large, convex, shiny, red-pigmented colonies on NSY agar plates (1.5 % agar), which reached a diameter of 5 mm after 18 days of incubation at room temperature (about 23 °C). No pronounced subsequent increase of colony diameter was observed. In liquid NSY medium (3 g l⁻¹, pH 7.2), the strain grew at 20 °C with a rate of 0.11±0.003 h⁻¹ (mean and SD of three parallels) equalling a generation time of 6.5 h and reached a maximum optical density at 575 nm (OD575) of about 0.38. In the exponential growth phase, the strain appeared with a rod-shaped morphology with cell length of 3–4 µm and cell widths of 0.6 µm. When the strain was cultivated in soft agar (1 g yeast extract l⁻¹, 0.1 g K2HPO4 l⁻¹, 2.0 g agar l⁻¹), swarming colonies reached a diameter of 30 mm within 3 days. Upon storage at about 23 °C for 3 weeks, the appearance of the culture changed from uniformly turbid to mycelia-like floccose in some spots. Light microscopic observation mainly revealed filamentous rods, 0.3 µm wide, and rather rare twisted spirals. The spirals typically had 4–7 right-handed turns and a diameter of 1–1.2 µm. Transitional states, like filaments seemingly starting to curl, were also observed. Scanning electron microscopic pictures confirmed the impression found at the light microscope, i.e. that there were no constrictions or separations visible along the spirals (Fig. 1). Local addition of 100 µl soil extract (prepared in water as described in DSMZ medium 80; www.dsmz.de/?id=441) near the colony edge promoted the formation of these spirals.

The spirals had high similarity to those observed in the aerial mycelium of species of the genus Streptomyces (phylum Actinobacteria). In order to exclude the possibility that
slowly growing streptomycetes were co-isolated with strain MWH-Nonnen-W8redT, the very cultures and spots from which the figures were taken were inoculated to media typically used for streptomycetes [DSMZ media 65 (GYM Streptomycyes Medium) and 987 (ISP2 Medium)], subjected to Gram staining (Fig. S1, available in the online Supplementary Material), and the 16S rRNA gene was sequenced. The filaments and spirals stained Gram-negative, the cultures did not grow on these media when incubated at 28°C for 7 days, and the 16S rRNA gene sequence did not show any difference to the sequences obtained by genome sequencing or previous Sanger sequencing of the gene. These results confirmed that the spirals are truly a morphological, and possibly a developmental, state of strain MWH-Nonnen-W8redT.

Similar spirals were reported previously from uncultured 'Spirobacillus cienkowskii' [1, 2], from Oligoflexus tunisiensis [7, 10] and from Bdellovibrio bacteriovorus [11]; however, the spirals observed in O. tunisiensis and Bdellovibrio bacteriovorus were not as densely packed as those in strain MWH-Nonnen-W8redT and in ‘Spirobacillus cienkowskii’.

Further phenotypic characterizations (Table 1) were performed as described previously [12, 13]. Assimilation of particular substances was tested by comparing growth on media with and without test substance [12]. Substrate-specific growth was determined by comparison of OD578 established in liquid one-tenth-strength NSY medium (0.3 g l⁻¹) with and without 0.5 g l⁻¹ test substrate, respectively. Differences of <10, 10–50 and >50 % of the OD obtained in the test treatments compared with the OD obtained without test substrate (i.e. in 0.3 g NSY l⁻¹ medium) were scored after 10 days of growth as no utilization (−), weakly positive utilization (w) and good utilization (+), respectively. The strain utilized D-mannose, D-glucose, L-proline, L-glutamate and L-alanine. Three other substances tested were only assimilated weakly (Table 1). These assimilation experiments recorded growth of the strain based on OD measurements. Additional substrate utilization tests were performed with BIOLOG GN2 MicroPlates, which detects utilization of substrates as electron donors by the subsequent reduction of a tetrazolium redox dye. These tests were performed as follows. Cells were suspended in distilled water, because initial tests revealed that this treatment gave higher scores compared with inoculation in a 0.17 % NaCl solution (the BIOLOG manual even suggests a 0.85 % NaCl solution). The plates were read after 48 h of incubation at 28°C. A threshold of 100 counts was set to evaluate a response as positive. The highest count observed was 232. These tests suggested that the strain is able to use α-D-glucose, α-ketobutyric acid, α-ketovaleric acid, succinic acid, L-asparagine, L-aspartic acid, L-hydroxyproline, L-proline, L-serine, L-threonine and inosine as electron donors. Note that these BIOLOG results and assimilation experiments based on growth of the strain tested are in partial contradiction regarding utilization of some substances. In addition to experiments by these two methods, substrate utilization tests with API20NE strips (bioMérieux) were performed according to the recommendations by the manufacturer. Interestingly, no substrate utilization nor any enzymic reactions were detected with the API20NE strips.

Additionally, enzymic activities of strain MWH-Nonnen-W8redT were tested by using API Zym strips (bioMérieux) incubated for four hours at 37°C. These experiments showed strong reactions for alkaline and acid phosphatases and intermediate reactions for C4 esterase, esterase lipase and leucine arylamidase. Test for oxidase and catalase activity performed as described previously [12] suggested that strain MWH-Nonnen-W8redT was oxidase-negative and weakly catalase-positive (Table 1).

It was observed that the phenotypic responses of the strain in growth experiments testing substrate utilization, temperature range for growth and salinity tolerance were not

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MWH-Nonnen-W8redT</th>
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<tbody>
<tr>
<td>Cell morphology</td>
<td>Pleomorphic</td>
</tr>
<tr>
<td>Cell length of rods (µm)</td>
<td>3.6</td>
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<tr>
<td>Cell width of rods (µm)</td>
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</tr>
<tr>
<td>Motility (soft agar)</td>
<td>+</td>
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<tr>
<td>Catalase/oxidase</td>
<td>w/−</td>
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<tr>
<td>Temperature range for growth (°C)</td>
<td>10–32 (w)</td>
</tr>
<tr>
<td>NaCl tolerance (% w/v)</td>
<td>0–1.0 (w)</td>
</tr>
<tr>
<td>Anaerobic growth</td>
<td>−</td>
</tr>
<tr>
<td>NSY medium</td>
<td>−</td>
</tr>
<tr>
<td>NSY enriched with nitrate</td>
<td>−</td>
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<tr>
<td>Assimilation of:</td>
<td></td>
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<tr>
<td>Glyoxylic acid</td>
<td>−</td>
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<tr>
<td>Glycolic acid</td>
<td>−</td>
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<tr>
<td>Acetic acid</td>
<td>w</td>
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<tr>
<td>Propionic acid</td>
<td>−</td>
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<tr>
<td>Oxaloacetic acid</td>
<td>−</td>
</tr>
<tr>
<td>Malonic acid</td>
<td>−</td>
</tr>
<tr>
<td>DL-Lactate</td>
<td>−</td>
</tr>
<tr>
<td>Fumaric acid</td>
<td>w</td>
</tr>
<tr>
<td>Citric acid</td>
<td>−</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>−</td>
</tr>
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<td>D-Mannose</td>
<td>+</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>+</td>
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<tr>
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<td>−</td>
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<tr>
<td>D-Sorbitol</td>
<td>−</td>
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<td>Glycine</td>
<td>w</td>
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<td>L-Glutamate</td>
<td>+</td>
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<tr>
<td>L-Methionine</td>
<td>−</td>
</tr>
<tr>
<td>Betaine</td>
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</table>

The assimilation data represent results of growth experiments performed as described previously [12]. −, Negative; +, positive; w, weakly positive.
reliable. Repetition of experiments yielded in some cases contradicting results. For instance, growth at 15 °C was in two experiments negative but positive in a third experiment. In all three experiments, controls incubated at room temperature (about 23 °C), which was also the incubation temperature of the culture used for inoculation of the experiments, were clearly positive, respectively. This lack of phenotypic reliability has to be considered in future comparative investigations including this strain.

The chemotaxonomic characterization of the strain included analyses of the composition of whole-cell fatty acids, polar lipids and quinones, as well as analysis of the peptidoglycan structure. The whole-cell fatty acid composition was analysed after growth at 28 °C on R2A and on NSY agar, respectively, by using an Agilent Technologies 6890 N instrument and the Microbial Identification System (MIDI) version 6.1 (results were evaluated against the instrument and the Microbial Identification System (MIDI) database) as described by Sasser [14]. Main compounds were iso-C15:0, C16:0, C17:0; however, the composition differed between biomass grown on the two different media (Table S1). A high number of 3-hydroxylated fatty acids were noticeable. In general, the fatty acid composition of strain MWH-Nonnen-W8redT differed significantly from that given for O. tunisiensis [7], in which C16:1ω5c and C16:1ω7c constituted 93% of the cellular fatty acids detected.

Polar lipids were extracted and analysed as described by Tindall [15, 16] based on the method by Bligh and Dyer [17]. This analysis revealed phosphatidylethanolamine and phosphatidylglycerol as the main components and a smaller proportion of an unknown lipid (Fig. S2). Extraction and analyses of respiratory quinones were also performed as described by Tindall [15, 16]; however, this analysis could not identify the quinones present. During development of the thin-layer chromatogram the compounds extracted showed an ascending height (rate) in between those of ubiquinones and menaquinones. The HPLC separation resulted in four peaks, but their retention times did not correspond to those of known ubiquinones or menaquinones. Thus, the quinones of the strain could be identified as neither ubi- nor menaquinones. The peptidoglycan structure of the strain was analysed according to the method of Schumann [18]. After preparation and hydrolysis of the peptidoglycan, meso-diaminopimelic acid was detected by GC/MS as expected in Gram-negatively-staining bacteria.

The genome of strain MWH-Nonnen-W8redT was sequenced and annotated. DNA used for genome sequencing was extracted from biomass grown in liquid NSY medium as described previously [19]. Two libraries were sequenced by an Illumina and a Roche system, respectively. A Long Jumping Distance (LJD) library of 8 kb fragment size was mate-pair sequenced on an Illumina MiSeq instrument, which resulted in 271 499 filtered reads with a mean length of 112 nt. Paired-end sequencing of a shotgun library on a GS FLX instrument by using Titanium chemistry resulted in 161 591 filtered reads with a mean length of 453 nt. A de novo hybrid assembly was conducted using an in-house pipeline (Eurofins Genomics) that incorporates the software tool newbler 2.9. This resulted in five scaffolds consisting of 41 contigs. Gap closure was performed by in silico analyses and by PCR amplification of gap regions and subsequent Sanger sequencing of amplicons. Thirteen gaps could be closed. The genome sequence obtained has a length of 3.51 Mbp and a DNA G+C content of 32.63 mol% and is characterized by a coverage of about ×30 (Table 2). The resulting genome sequence was annotated using the (Integrated Microbial Genomes/Expert Review) IMG/ER annotation pipeline [20]. Additionally, the genome was annotated by using the NCBI pipeline for prokaryotic genomes and deposited in DDBJ/EMBL/GenBank under the accession numbers CP017834–CP017838.

The genome of strain MWH-Nonnen-W8redT putatively encodes 3049 protein and 53 RNA genes. It consists of five scaffolds representing one chromosome, two putative conjugative plasmids and two putative prophages. The four smaller scaffolds share a small size of about 40 kbp each. The putative conjugative plasmids both encode a relaxase, a type IV coupling protein, a type IV secretion system putatively involved in transfers of the two conjugative plasmids, respectively, and a DNA topoisomerase. The putative prophages both encode terminases and oligoribonucleases; however, only one of the two putative prophages encodes a substantial number of genes annotated as putative phage genes.

The chromosome of the strain encodes five copies of ribosomal operons. These operons could be assembled but contain gaps of unknown sequences located downstream of the 16S rRNA genes, respectively. Genes 2653193881–2653193883 (IMG Gene ID) encode a putative non-ribosomal peptide synthetase/polyketide synthase system. Annotations of these three genes hint on synthesis of

<table>
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<tr>
<th>Scaffold</th>
<th>Type</th>
<th>Accession number</th>
<th>Size</th>
<th>No. genes</th>
<th>DNA G+C content (mol%)</th>
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<tr>
<td>1</td>
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<td>CP017834</td>
<td>3.34 Mbp</td>
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<td>42.2 Kbp</td>
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<td>Putative conjugative plasmid</td>
<td>CP017836</td>
<td>37.0 Kbp</td>
<td>42</td>
<td>29</td>
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</table>

Table 2. Genome characteristics of strain MWH-Nonnen-W8redT
products with putative antimicrobial activity (lichenysin-like substances). Two other putative non-ribosomal peptide synthetases are encoded by genes 2653195819 and 2653195162 (IMG Gene ID), which are both annotated as a bacitracin synthase. All these genes encode large proteins of 700–2021 amino acids. At all three loci with putative non-ribosomal synthetase genes, open reading frames encoding putative drug/metabolite transporters are present nearby. Furthermore, the genome encodes four giant genes of >10000 bp, which are all annotated as fibronectin type 3 domain-containing protein (IMG Gene IDs 2653194608, 2653194165, 2653195410 and 2653194609).

Besides the above-mentioned two plasmid-encoded type IV secretion systems, the genome encodes on its chromosomes a Sec-pathway (general secretion route) and a twin-arginine translocation pathway, which both mediate the secretion of proteins across the cytoplasmic membrane. Some other chromosomally encoded genes seem to belong to a type II secretion system; however, it seems that the set of genes necessary for synthesis of a functional type II system is incomplete. No genes potentially contributing to type III or type VI secretion systems were annotated. Regarding presence and absence of chromosomal genes encoding secretion systems, the gene content of strain MWH-Nonnen-W8redT is quite similar to those of *Bdellovibrio bacteriovorus* HD 100T and *Halobacteriovorax marinus SJ*T, but the latter two lack plasmid-related type IV systems. By contrast, strains of the genus *Myxococcus* usually encode both a type III and a type VI secretion system. See below for the phylogenetic relationships of the taxa mentioned with strain MWH-Nonnen-W8redT.

Relating to the phylogenetic relationships suggested below between strain MWH-Nonnen-W8redT and members of the genus *Oligoflexus* and the order *Bdellovibrionales*, it is interesting that the former two taxa do not possess the majority of the 59 genes present in all genome-sequenced members of the order *Bdellovibrionales* lacking in other previously investigated bacteria [21]. For instance, BLASTp searches resulted in hits to the genome of strain MWH-Nonnen-W8redT only for nine of the 59 query protein sequences; however, all the resulting alignments were characterized by identity values of ≤32 % and E values of ≥e-14. Interestingly, the genome of strain MWH-Nonnen-W8redT lacks homologues of the *hit* locus, which is a conserved region in genomes of members of the genera *Bdellovibrio* and *Halobacteriovorax* known to encode functions involved in the predatory lifestyle of these bacteria [22].

Regarding the above-mentioned motility of the strain and the potential virulence (water flea) of the strain discussed below, it is worth mentioning that its genome contains genes putatively encoding the synthesis and use of flagella, as well as putative chemotaxis genes.

Genome comparisons based on average nucleotide identity (ANI) analyses [23] of the MWH-Nonnen-W8redT genome with the most closely related type strains available (see below), i.e. with *O. tunisiensis* Shr3T [24], the type strains of species of the genus *Bdellovibrio* [25, 26], *H. marinus SJ*T [22] and representative strains of *Bacteriovorax* spp. [21], resulted in quite low values of 66–69 % ANI, which suggests only distant phylogenetic relationships between strain MWH-Nonnen-W8redT and these taxa. In all three comparisons, these results obtained by using the IMG system [20] are based on alignment fractions of only about 1–2 % of the genome sequences. Two-way average amino acid identity (AAI) values calculated with the AAI calculator [27] for those genomes resulted in AAI values of about 35–38 %, respectively. These results are based on alignments of >40 % of the proteins encoded by the genome of MWH-Nonnen-W8redT. ANI and AAI results both suggest only distant phylogenetic relationships of strain MWH-Nonnen-W8redT with the taxa compared. It should also be mentioned that strain MWH-Nonnen-W8redT and the reference taxon with the most similar 16S rRNA gene sequence, i.e. *Vulgatibacter incomptus* DSM 27710T, even share an ANI value of 79.6 % but the alignment fraction is less than 0.1 % of the genome sequences.

The DNA G+C value of the MWH-Nonnen-W8redT genome of 32.6 mol% is exceptionally low for a proteobacterium with a genome size of >3 Mbp (Fig. 2). Among the 14351 genomes of cultured members of the phylum *Proteobacteria* (environmental genomes were excluded) available in the IMG system [20] at the time of analysis (June 2016) characterized by genome sizes ≥3 Mbp, only 11 genomes found with DNA G+C contents less than 35 % (Fig. 2). Among these 11 taxa, no member of the current order *Bdellovibrionales* or the current class *Oligoflexia* are found. Interestingly, six genomes currently classified by the IMG system as ‘*Bacteriovorax*’ strains (including *H. marinus SJ*T),

![Fig. 2. Frequency distribution of DNA G+C values of genomes of members of the phylum *Proteobacteria* available in the Integrated Microbial Genomes (IMG) system characterized by genome sizes of ≥3 Mbp. Only four and eight (including MWH-Nonnen-W8redT) genomes possess DNA G+C values in the range of 25–30 and 30–35 mol%, respectively.](image-url)
which should be considered as members of the new order Bacteriovoracales ord. nov. proposed below, possess DNA G+C values in the range of 35–40 %. However, the DNA G+C values of the genomes of other members of the current order Bdellovibrionales, e.g. of species of the genus Bdellovibrio, as well as of O. tunisiensis Shr3T, are higher than 40 %. Thus, a DNA G+C content of less than 40 % is no common feature of the revised class Oligoflexia proposed below. Genomes with sizes smaller than 3 Mbp were excluded from these analyses, because genomes shaped by reductive genome evolution usually possess reduced DNA G+C values. However, of the 3002 proteobacterial genomes with sizes of less than 3 Mbp, 75 % possess DNA G+C values higher than the value of MWH-Nonnen-W8redT.

We searched for other exceptional genomic or genetic traits of strain MWH-Nonnen-W8redT. An interesting feature is the lack of the diagnostic amino acid sequence GGKH in the alanyl-tRNA synthetase, which is assumed to be present in this protein in all members of the phylum Proteobacteria [28]. A systematic screening of 6212 proteobacterial genomes available in the IMG system revealed that strain MWH-Nonnen-W8redT is quite exceptional in this trait. For this screening, all genomes assigned to the classes Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Epsilonproteobacteria, ‘Zetaproteobacteria’, Acidithiobacillia and Oligoflexia, respectively, were considered, but due to the large number of genomes assigned to the class Gammaproteobacteria only those of this class with the status ‘finished’ were included in the analysis. After exclusion of low-quality genomes completely lacking an annotation of the alanyl-tRNA synthetase gene or putatively containing only an incomplete gene, 5808 genomes remained for the further analysis. Of these genomes, 34 encoded alanyl-tRNA synthetases with substitutions in the signature sequence, and in nine genomes the gene possessed a complete deletion of the four-amino-acid signature sequence. Interestingly, all those nine genomes are classified by the IMG system as ‘Bacteriovorax’, which includes again H. marinus SJ1. By contrast, the signature sequence was found among all other genome-sequenced strains currently classified as members of the order Bdellovibrionales, as well as in O. tunisiensis Shr3T. Twenty-two of the 34 genomes with substitutions in the signature sequence are currently classified as members of the class Deltaproteobacteria, which also includes the obviously misclassified Vampirovibrio chlorellavorus [29].

Furthermore, we tested if those taxa currently classified as members of the class Deltaproteobacteria, but proposed by us to be assigned to the class Oligoflexia (see below), could be distinguished from the other members of the class Deltaproteobacteria by the copy number of the ribosomal protein S1 gene. Karlin et al. [30] suggested that delta-proteobacterial genomes encode two ‘giant’ S1 ribosomal protein genes, while other bacteria encode only a single copy. We analysed 183 genomes of bacteria classified at the time of investigation (spring 2016) as members of the class Deltaproteobacteria by using the IMG system. Genomes of strains not classified at the species level, as well as ‘environmental genomes’ (metagenomic assemblies and single-cell genomes) were excluded. Of the members of the class Deltaproteobacteria investigated, 46.4 % encode one, 53.0 % encode two and 0.6 % encode three genes annotated as ribosomal protein S1 genes (COG 0539). Strain MWH-Nonnen-W8redT, as well as none of the genomes currently representing the order Bdellovibrionales, encode two copies of the gene; however, O. tunisiensis Shr3T encodes two non-identical genes with this annotation. Obviously, the copy number of the ribosomal protein S1 gene is not a homogeneous feature among bacteria currently classified as members of the genus Deltaproteobacteria and is also not suitable for distinguishing those from other members of the phylum Proteobacteria.

For gaining a first hint on the phylogeny of strain MWH-Nonnen-W8redT, comparative analyses of the 16S rRNA gene were performed. The strain encodes five copies of this ribosomal gene, all sharing identical sequences. BLAST searches revealed that type strains with the most similar gene sequences belong to various classes of the phylum Proteobacteria. Surprisingly, no type strains sharing a 16S rRNA gene sequence similarity of more than 85 % with the new isolate could be found. However, inclusion of non-type-material in analyses resulted in much higher sequence similarity values. ‘Spirobacillus cienkowskii’, an uncultured pathogen of water flea (Daphnia spp.), which was described by Élie Metchnikoff almost 130 years ago [1] and rediscovered a few years ago by Rodrigues and colleagues [2], shares a 16S rRNA gene sequence similarity of 96 %. The morphology [1, 2] of this so-far uncultured bacterium, as well as its 16S rRNA and gyrase B subunit gene sequences [2] have been described.

For reconstruction of the phylogenetic position of the strain, multilocus protein trees with a large set of proteins extracted from a phylogenetically broad set of reference taxa were calculated. Overall, our phylogenetic analyses followed the strategy described by Williams and Kelly [31]. We selected a set of genome-sequenced reference strains representing the whole phylogenetic width of the phylum Proteobacteria, as well as a couple of representatives of other phyla. We tried to optimize the set of reference strains for high proportions of type strains, high proportions of high-quality genomes and a balanced taxonomic distribution across the phylum Proteobacteria. We also included the genome of O. tunisiensis Shr3T [24], which represents the most recently described class of the phylum Proteobacteria [7]. We screened each selected genome for the presence of the 98 protein families used in the analyses by Williams and Kelly [31] previously. If a family was lacking in one or more genomes, we rejected either the genome (if genome sequences of close relatives were available) or the protein family from the further analyses. These analyses were performed by using the IMG/ER (Expert Review) system [20]. Protein families were identified by their COG (Clusters of Orthologous Groups) classification, and families represented by more than one
gene from the same genome in a COG category were usually rejected. Finally, the set of reference strains consisted of 84 strains (basic reference set, Table S2) and the set of protein families consisted of 74 COGs (Table S3). In a second analysis step, we enriched the set of reference strains for members of the phylum Acidobacteria and strains affiliated with the deltaproteobacterial order Bdellovibrionales to a total number of 93 reference strains (extended taxon set; Tables S2 and S4).

Protein sequences were extracted from the genomes, and separate alignments were established for each COG by using MUSCLE [32] implemented in the software MEGA7 [33]. Alignments were trimmed, and protein sequences of each reference strain were concatenated. These alignments were masked with Gblocks V0.91b [34] in order to reduce phylogenetic noise potentially caused by unreliable aligned regions. Masking was optimized by stepwise relaxation of the masking and comparison of bootstrap results of trees calculated by RAxML [35] with the differently masked alignments. Four different masking settings were tested, which resulted in alignments consisting of 32 to 66% of the positions in the primary alignment. The mean bootstrap values of the particular RAxML trees increased with increasing relaxation of the masking criteria. Finally, in contrast to the analyses performed by Williams and Kelly [31], quite relaxed criteria for masking were selected, which included, for instance, a high gap tolerance (setting ‘all’). The protein alignment used for reconstruction of phylogenetic trees consisted of 20,950 alignment positions. Treeing was performed with the RAXML, MrBayes (version 3.2.1, [36]) and neighbour-joining (MEGA7) algorithms.

The trees calculated with these three different algorithms placed strain MWH-Nonnen-W8redT consistently in a branch formed by species of the genus Bdellovibrio, H. marinus and O. tunisiensis (Fig. 3). These trees confirm the status of O. tunisiensis Shr3T as the type of its own class of the phylum Proteobacteria [7]. However, these trees also suggest that the representatives of the order Bdellovibrionales included are, in contrast to their current classification, not affiliated with the class Deltaproteobacteria. In order to test the phylogenetic position of the order Bdellovibrionales, the set of reference strains was expanded by addition of four more strains currently classified as members of this order, as well as addition of some more taxa affiliated with the phylum Acidobacteria. This expansion of the taxon set did not change the formation of a well-bootstrap-supported branch consisting of the genus Oligoflexus, MWH-Nonnen-W8redT and members of the order Bdellovibrionales (Fig. S4) except Vampirovibrio chlorellavorus [37, 38]. The reconstructed phylogenetic position of Vampirovibrio chlorellavorus confirms that this strain neither belongs to the order Bdellovibrionales nor to the phylum Proteobacteria, but is affiliated with the phylum ‘Candidatus Melainabacteria’ [39]. This candidatus phylum represents a sibling phylum to the phylum Cyanobacteria [39, 40].

In contrast to the tree based on the primary taxon set, the tree based on the extended set places the monophyletic lineage formed by the genus Oligoflexus, MWH-Nonnen-W8redT and the order Bdellovibrionales within the class Deltaproteobacteria (Fig. S4). Importantly, the affiliation of this lineage with the class Deltaproteobacteria lacks any bootstrap support, while the separate phylogenetic positioning suggested by the previous multi-protein tree (Fig. 3) and 16S rRNA gene trees ([7], Figs S5 and S6) clearly suggest a phylogenetic position outside of the class Deltaproteobacteria.

Another major difference between the two multi-protein trees is the position of the branch representing the phylum Acidobacteria. This group appeared in the first analysis between the class Alphaproteobacteria and the major branch of the class Deltaproteobacteria but the extension of the taxon set shifted the position to between the classes Deltaproteobacteria and the Epsilonproteobacteria (Fig. S4).

The results of the phylogenetic analyses performed have diverse taxonomic implications. They confirm the previously revealed paraphyletic nature of the phylum Proteobacteria. Independently established trees suggest that taxa representing the class Epsilonproteobacteria are more closely related to taxa affiliated to other phyla than the phylum Proteobacteria [31, 41–43] (Figs 3 and S4). The class Epsilonproteobacteria and the deltaproteobacterial order Desulforellales appear to be more distantly related to the major part of the phylum Proteobacteria than the phylum Acidobacteria ([31], Fig. 3). Besides the currently single species class Oligoflexus, which cannot be evaluated for monophyly, all other proteobacterial classes but the class Deltaproteobacteria appear to be monophyletic clades. Interestingly, the polyphyletic nature of the class Deltaproteobacteria was also suggested by analyses based on 16S rRNA gene sequences [26, 44]. Furthermore, it is obvious that the current class Deltaproteobacteria differs from all other classes of the phylum Proteobacteria in its phylogenetic breadth. Obviously, revisions of the classes Deltaproteobacteria and Epsilonproteobacteria are required regarding membership of subgroups or taxonomic rank; however, these tasks are beyond the scope of this study. Nonetheless, the appropriate classification of strain MWH-Nonnen-W8redT proposed below requires a revision of the classification of the order Bdellovibrionales. Furthermore, a reclassification of the species Vampirovibrio chlorellavorus in a new Candidatus phylum or class ‘Melainabacteria’ [29], would be advisable. However, since no viable culture of the type strain is available [29] and no cultured representative of ‘Candidatus Melainabacteria’ is available yet, a revision of the classification of Vampirovibrio chlorellavorus has to be postponed until a member of this clade can be cultivated.

Does MWH-Nonnen-W8redT represent ‘Spirobacillus cienkowskii’? Strain MWH-Nonnen-W8redT is, regarding cell morphology, morphological variability and pigmentation, very similar to ‘Spirobacillus cienkowskii’ characterized by
Metcninkoff in 1889 [1]. Metcninkoff observed and described the life cycle of ‘Spirobacillus cienkowskii’ in infected Daphnia. He reported various morphological forms, including rods, spiralae and filaments. Appearance of such ‘Spirobacillus cienkowskii’ morphotypes in infected Daphnia spp. was confirmed by Rodrigues and colleagues.
By using fluorescent in situ hybridization (FISH) probes specific for ‘Spirobacillus cienkowskii’, they demonstrated that all these morphotypes belong to this taxon. We observed the same morphotypes in cultures of MWH-Nonnen-W8red\textsuperscript{T} including the unusual densely coiled spirals (compare Figs 1 and 2d of Rodrigues et al. [2]); however, formation of spirals occurred only under specific cultivation conditions. Obviously, both taxa share quite unusual morphologic features and a morphological plasticity. Importantly, very similar morphologies including spirillae, filaments, curved rods and spherical cells were observed for ‘Spirobacillus cienkowskii’ (Fig. 4). By contrast, only the latter two taxa seem to share pigmentation by a red or pink–red carotenoid. Green detected a carotenoid in ‘Spirobacillus cienkowskii’ [45], and the genome of MWH-Nonnen-W8red\textsuperscript{T} encodes genes putatively enabling this organism to synthesize at least the red-pigmented carotenoid lycopene (Table S5). These phenotypic similarities between strain MWH-Nonnen-W8red\textsuperscript{T} and ‘Spirobacillus cienkowskii’ are contrasted by differences in 16S rRNA gene and gyrB sequences. The 16S rRNA gene sequence of ‘Spirobacillus cienkowskii’ determined by Rodrigues et al. [2] and the sequence of MWH-Nonnen-W8red\textsuperscript{T} share a similarity of only 95.9 % (57 bp different) and, importantly, are also distinguished by five nucleotide insertions at two sites of the ‘Spirobacillus cienkowskii’ gene. The gyrB gene of the two taxa shared only a nucleotide sequence similarity of 82 % (protein identity 92 %) but the gyrB genes of both taxa share very similar DNA G+C contents of about 36 mol%. Interestingly, Rodrigues and colleagues found no differences in the 16S rRNA gene sequences across a couple of European ‘Spirobacillus cienkowskii’ populations but a sequence difference of 1 % between European and North American populations [2]. All these sequences were obtained from infected Daphnia spp. including at least three different host species. The very high sequence similarity among ‘Spirobacillus cienkowskii’ populations across host species and continents makes it unlikely that MWH-Nonnen-W8red\textsuperscript{T} represents an ‘Spirobacillus cienkowskii’-like pathogen of Daphnia spp.

Other 16S rRNA gene sequences sharing similarities of >96 % with the gene of strain MWH-Nonnen-W8red\textsuperscript{T} (Figs S5 and S6) mainly represent aquatic bacteria of unknown lifestyle [3–6]. Some of these organisms dwelled in surface freshwater habitats like Yellowstone Lake [6], while other sequences were obtained from a peat bog [4] or a subsurface water pool [5]. Because it is unlikely that in all those habitats daphnids are present, it can be assumed that at least some organisms sharing 16S rRNA gene sequence similarities of ≥96 % with ‘Spirobacillus cienkowskii’ do not represent obligate pathogens of Daphnia spp.

![Fig. 4. Revised taxonomy of the class Oligoflexia. The neighbour-joining tree shown was calculated with almost-complete 16S rRNA gene sequences of bacteria proposed to be classified in the previously monotypic class Oligoflexia. Alignment positions with gaps in any sequence were completely omitted for the tree calculation, which resulted in an alignment length of 1343 positions. Phylogenetic distances were calculated by using the Tamura 3-parameter substitution model. Sequences of taxa not affiliated with the phylum Proteobacteria were used as an outgroup (not shown). Bootstrap values obtained with the neighbour-joining, the maximum-likelihood and the maximum-parsimony methods (1000, 100 and 100 replications, respectively) are indicated, respectively. Note that this 16S rRNA gene tree differs regarding the position of the revised order Bdellovibrionales from the multi-protein trees calculated (Figs 4 and S3); however, the responsible node is only weakly supported in the 16S rRNA gene sequence tree. The branching order of the neighbour-joining and maximum-likelihood trees calculated is identical. Bar, 0.05 substitutions per nucleotide position.

Bacteriovoracales ord. nov.

Oligoflexales

Silvanigrellales ord. nov.

Bdellovibrionales

\[2\]
Two experiments were performed in order to test if MWH-Nonnen-W8red is able to infect *Daphnia cf. pulex*. The species *D. pulex* was reported to be susceptible to infections by *'Spirobacillus cienkowskii'* [2]. In a first experiment, daphnids were challenged with $1.5 \times 10^6$ MWH-Nonnen-W8red cells ml$^{-1}$. Besides the bacteria added, the daphnids also received algae (*Cryptomonas* sp. 26.80) as food. The batch cultures (40 ml, six replicates) containing the daphnids were fed every 2 to 3 days with a mixture containing algae and the bacteria tested. Throughout the experiment, which lasted for 23 days, the food cocktail added contained 100 times more bacterial carbon (MWH-Nonnen-W8red) than algal carbon (*Cryptomonas* sp.). Two controls were included in the experiment, both with six replicates (40 ml each). The first control (*Cryptomonas* only) received no bacteria but the same amount of algal carbon as the test treatments. The second control received instead of MWH-Nonnen-W8red the terrestrial bacterium *Cupriavidus basilensis* DSM 11853 [46] and the algal food. The total amount of carbon, as well as the carbon ratio of algae to bacteria (1 : 100) was identical in the two treatments receiving bacteria. The total number of daphnids and the number of offsprings were counted during the experiment at 18 days. Special attention was paid to the appearance of red-coloured daphnids and dead daphnids. Interestingly, the daphnids grew better in the control treatment without added bacteria; however, in the treatment which received strain MWH-Nonnen-W8red, the daphnids grew better than in the treatment with *Cupriavidus basilensis* DSM 11853 (Fig. S7). Red-pigmented daphnids or other hints of a bacterial infection were not observed in any replicate of all three treatments. A second experiment challenging daphnids with higher concentrations of MWH-Nonnen-W8red cells was conducted in order to test if infections occur at higher doses. Four parallel treatments receiving 3, 6, 9 and $12 \times 10^6$ MWH-Nonnen-W8red cells ml$^{-1}$ were established. All treatments received the same amount of algal food and were fed in the same way during the experiment. Again, infected daphnids were not observed in any of the four treatments.

In general, the two experiments did not result in any hint of a pathogenic potential of strain MWH-Nonnen-W8red regarding *Daphnia cf. pulex*; however, we cannot really exclude that the strain would be able to infect daphnids if an appropriate host or appropriate infection conditions would be given. We note that a pathogenic potential of strain MWH-Nonnen-W8red could not be demonstrated so far.

**PROPOSAL OF THE NOVEL SPECIES SILVANIGRELLA AQUATICA GEN. NOV., SP. NOV. AND REQUIRED TAXONOMIC REVISIONS**

The large phylogenetic distance of strain MWH-Nonnen-W8red from any described species does not leave any doubt that this strain represents a novel species. According to the phylogenetic trees obtained, *O. tunisiensis* and members of the order *Bdellovibionales* represent the most closely related species currently described. We propose to establish for the strain investigated here the novel genus and species *Silvanigrella aquatica* gen. nov., sp. nov. and to place it in the class *Oligoflexia* [7] of the phylum *Proteobacteria* [47]. The currently available characterization of *'Spirobacillus cienkowskii'* is too superficial to determine if strain MWH-Nonnen-W8red and those pathogens of daphnids should be placed in the same genus. Because of lack of evidence for pathogenicity in strain MWH-Nonnen-W8red and because of the inappropriateness of the name *bacilllus* for a proteobacterium, we refrain from proposing *'Spirobacillus'* as the genus name to accommodate the newly described strain.

The 16S rRNA gene sequence similarity value of less than 82% between *O. tunisiensis* and strain MWH-Nonnen-W8red provides strong evidence for placement of the two strains in distinct orders [43]. Therefore, we propose to establish for *Silvanigrella aquatica* gen. nov., sp. nov. the new family *Silvanigrellaceae* fam. nov. to be placed in the new order *Silvanigrellaes* ord. nov. of the class *Oligoflexia*. Furthermore, the multi-protein (Fig. 3) and 16S rRNA gene phylogenies (Fig. 4) presented here suggest the transfer of the order *Bdellovibrionales* from the class *Deltaproteobacteria* to the class *Oligoflexia*. A rather isolated position of the genus *Bdellovibrio* or the order *Bdellovibionales* within the class *Deltaproteobacteria* was shown previously [22, 48], and lack of bootstrap support for placement in this class was shown previously [22]. Interestingly, the multi-protein tree calculated by Williams and Kelly [31], which did not include the genus *Oligoflexus*, and the multi-protein tree presented here (Fig. 3) differ in bootstrap support for the class *Deltaproteobacteria*. While the tree lacking the genus *Oligoflexus* integrated the genus *Bdellovibrio* in the class *Deltaproteobacteria* but lacked bootstrap support for this class (39%), our tree excludes the order *Bdellovibionales* from the class *Deltaproteobacteria* and supports the remaining class with high bootstrap support in trees calculated with two out of three algorithms (Figs 3 and S4). Only the neighbour-joining algorithm did not result in a sufficient bootstrap support. Based on phylogenetic analyses of multi-protein alignments, the transfer of the order *Bdellovibionales* [49] from the class *Deltaproteobacteria* [50] to the class *Oligoflexia* [7] is proposed.

According to phylogeny and because none of the type species of the genera *Bacteriovorax*, *Peredibacter* and *Halobacteriovorax* share 16S rRNA gene sequence similarities of more than 82% with the type species of the genus *Bdellovibrio* we propose to establish the new order *Bacteriovoracales* ord. nov. for those three genera (Fig. 4). Finally, based on phylogenetic analyses of 16S rRNA gene sequences (Fig. 4), the transfer of the family *Pseudobacteriovoracaceae* McCaulley *et al.* 2015 [44] from the order *Bdellovibionales* Garrity *et al.* 2006 [51] to the order *Oligoflexales* Nakai *et al.* 2014 [7] is proposed. The description of the family remains as given by McCaulley *et al.* 2015 [44].
DESCRIPTION OF SILVANIGRELLA GEN. NOV.

Silvanigrella [Sil.va.ni.grel.la] N.L. fem. dim. n. Silvanigrella named after Silva nigra the Latin geographic name of the Schwarzwald (Black Forest) mountains located in the southwest of Germany].

The description of the genus is based on the polyphasic characterization of the type strain of the sole species proposed to be affiliated currently with this genus. Features probably characterizing other strains affiliated with this genus are as follows: Gram-stain-negative, pleomorphic cell morphology, aerobic chemo-organoheterotrophs, red pigmentation.

The genus is a member of the class Oligoflexia [7] of the phylum Proteobacteria [47]. The type species is Silvanigrella aquatica sp. nov.

DESCRIPTION OF SILVANIGRELLA AQUATICA SP. NOV.

Silvanigrella aquatica (aqua.ti.ca. L. fem. adj, aquatica living, growing, or found in the water, aquatic).

Apart from the characters given for the genus, the species is characterized as follows. Weakly catalase-positive, oxidase-negative. Cells are motile. Cell morphology is pleomorphic, ranging from short and large rod-shaped cells to filamentous morphology and formation of densely coiled spirals. Red pigmentation. Aerobic chemo-organoheterotroph, anaerobic growth is observed neither on standard NSY medium nor on NSY medium enriched with nitrate. Temperature range is 10 °C to 32 °C, and the salt tolerance is up to 1.0 % NaCl (w/v); however, growth at 1.0 % salinity is quite weak. Assimilates D-mannose, D-glucose, L-proline, L-glutamate and L-alanine. Weakly positive assimilation of acetate, fumarate and glycine. No assimilation of glyoxylate, glycolate, glycerol, propionate, oxaloacetate, malonate, lactate, citrate, D-xylene, D-fucose, D-sorbitol, L-methionine or betaine. Main fatty acids are iso-C₁₅:₀, anteiso-C₁₅:₀, feature 3 including C₁₆:₁ω7c and iso-C₁₄:₀5-OH, C₁₆:₀, C₁₇:₁ω8c and C₁₇:₀. Main polar lipids are phosphatidylethanolamine and phosphatidylglycerol. Contains unidentified quinones; known ubi- and menaquinones could not be detected.

The type strain is MWH-Nonnen-W8red T (=DSM 23856 T =CCUG 58639 T), which is the only strain investigated so far. The type strain was isolated from a water sample obtained from a freshwater lake located in the Black Forest Mountains, Germany. The genome of the type strain has a size of about 3.5 Mbp and a DNA G+C content of 32.6 mol %. The genome sequence has been deposited in DDBJ/EMBL/GenBank under the accession numbers CP017834–CP017838.

DESCRIPTION OF SILVANIGRELLACEAE FAM. NOV.

Silvanigrellaceae (Sil.va.ni.grel.la.ce.œ. N.L. fem. dim. n. Silvanigrella type genus of the family; suff. -aceae ending to denote a family; N.L. fem. pl. n. Silvanigrellaceae the family of the genus Silvanigrella).

The description is the same as for the genus Silvanigrella. The type genus is Silvanigrella gen. nov.

DESCRIPTION OF SILVANIGRELLALES ORD. NOV.

Silvanigrellales (Sil.va.ni.grel.la.œ. N.L. fem. dim. n. Silvanigrella type genus of the order; suff. -ales ending to denote an order; N.L. fem. pl. n. Silvanigrellales the order of the genus Silvanigrella).

The description is based on phylogenetic analyses of 16S rRNA gene sequences. Includes the family Silvanigrellaceae and ‘Candidatus Turabacter’ [52], as well as undescribed taxa or taxa without validly described names, which are predominantly found in freshwater systems. This includes strains isolated from the skin of an amphibian [3]; the pathogen of water flea, ‘Spirobacillus cienkowski’ [2]; the non-aquatic isolate GLA1 (accession number KF246685) from a human lymph node aspirate (Humrighouse, Whitney and McQuiston, Genbank deposition); uncultured bacteria found in surface freshwater systems like natural [6] and artificial lakes [53], wetlands like a peat bog system [4], or a subsurface epiphreatic pool in a karst cave [5]. The type genus is Silvanigrella gen. nov.

DESCRIPTION OF BACTERIOVORACALES ORD. NOV.

Bacteriovoracales (Bac.te.ri.o.vo.ra.ca.œ. N.L. masc. n. Bacteriovorax type genus of the family; suff. -ales ending to denote an order; N.L. fem. pl. n. Bacteriovoracales the order of the genus Bacteriovorax).

Encompasses the families Bacteriovoraceae Davidov and Jurkevitch 2004 [54] and Halobacteriovoraceae Koval et al. 2015 [55]. The description of the order is based on the descriptions of the families. This order is composed of Gram-negative, vibroid bacteria. They are obligate or facultative predators of various Gram-negative bacteria. The type genus is Bacteriovorax [56]. The order belongs to the class Oligoflexia.

EMENDED DESCRIPTION OF THE ORDER BDELLOVIBRIONALES GARRITY ET AL. 2006

Bdellovibrioales (Bdel.lo.vi.bri.o.œ. N.L. masc. n. Bdellovibrio type genus of the order; suff. -ales ending denoting an order; N.L. fem. pl. n. Bdellovibrioales the order of the genus Bdellovibrio).

Includes solely the genera Bdellovibrio Stolp and Starr 1963 [57], Micavibrio Lambina et al. 1989 [58] and Vamptiovibrio Gromov and Mamkayeva 1989 [59], i.e. members of the illegitimate family ‘Bdellovibrioaceae’ [59]. The description of the order Bdellovibrioales remains as given by Garrity et al. [49] except for the exclusion of the families Bacteriovoracaceae,
Halobacteriovoraceae and Pseudobacteriovoraceae. The type genus is Bdellovibrio Stolp and Starr 1963 [57].

**EMENDED DESCRIPTION OF THE ORDER OLIGOFLEXALES NAKAI ET AL. 2014**

Oligoflexales (O.li.go.fle.xa.les. N.L. masc. n. Oligoflexus type genus of the order; sufli. -ales ending to denote an order; N.L. fem. pl. n. Oligoflexales the order of the genus Oligoflexus).

Encompasses the families Oligoflexaceae and Pseudobacteriovoraceae. The description is based on the descriptions of the genera Oligoflexus [7] and Pseudobacteriovorax [44]. Gram-stain-negative, chemo-organoheterotrophs; obligate aerobes; pleomorphic including filamentous stages. The type genus is Oligoflexus.

**EMENDED DESCRIPTION OF THE CLASS OLIGOFLEXIA NAKAI ET AL. 2014**

Oligoflexia (O.li.go.fle.xi.a. N.L. masc. n. Oligoflexus type genus of the type order of the class; suffli. -ia ending to denote a class; N.L. fem. pl. n. Oligoflexia the class of the order Oligoflexales).

The class is described on the basis of a phylogenetic analysis of 16S rRNA gene sequences presented by Nakai et al. [7], and additionally includes the monophyletic lineage formed by 'Spirobacillus cienkowiskii' (accession number of the 16S rRNA gene sequence EU220836) and related cultured and uncultured bacteria (compare Fig. 3 of Nakai et al. [7]). Includes the orders Oligoflexales, Bdellovibrionales, Bacteriovoraceales and Silvanigrellales. The type order is Oligoflexales.

**EMENDED DESCRIPTION OF THE CLASS DELTAPROTEOBACTERIA KUEVER ET AL. 2006**

Deltaproteobacteria (Del.ta.pro.te.o.bac.te.ri.a. Gr. n. delta name of the fourth letter of the Greek alphabet; Gr. or L. n. Proteus Greek god of the sea, capable of assuming many different shapes; N.L. n. bacter a rod; sufli. -ia ending to denote a class; N.L. neut. pl. n. Deltaproteobacteria).

The description of the class Deltaproteobacteria remains as given by Kuever et al. [50] with the exception that the order Bdellovibrionales with its current taxa 'Bdellovibrionaceae', Bacteriovoraceae, Halobacteriovoraceae and Pseudobacteriovoraceae are excluded from the classis.

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

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**Ethical statement**
The presented study does not include any experimental work with humans or vertebrates.

**References**


