Silvanigrella aquatica gen. nov., sp. nov., isolated from a freshwater lake located in the Black Forest, Germany, description of *Silvanigrellaceae* fam. nov., *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 *Oligoflexiales*

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ABSTRACT

The unusual chemoorganoheterotrophic proteobacterial strain MWH-Nonnen-W8red^T 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 *Proteobacteria*. Whole genome sequencing resulted in a genome size of 3.5 Mbp and revealed a quite low 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 *Deltaproteobacteria* to the *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 *Oligoflexiales*. We propose to establish for strain MWH-Nonnen-W8red^T (DSM 23856^T =CCUG 58639^T) the new 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-W8red^T 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. BLAST searches against sequences of type material revealed that the top hits (November 2016) represent type strains belonging to various classes of *Proteobacteria*. The best hit, *Vulgatibacter incomptus* DSM 27710^T (*Deltaproteobacteria*), shared a 16S rRNA 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 similarity of 96%. Other taxonomically unclassified cultured and uncultured bacteria even share 97-99% 16S rRNA gene similarities [3-6]. According to Nakai and colleagues, who recently described the new class *Oligoflexia* of the phylum *Proteobacteria*, 'Spirobacillus cienkowskii' and related strains may represent a novel class of *Proteobacteria* [7].

We characterized strain MWH-Nonnen-W8red^T by following the polyphasic approach and included genome sequencing and comparative analysis of the annotated genome sequence of the strain. Based on the obtained results, we propose that this strain represents a new species, genus, family, and order affiliated with the class *Oligoflexia* Nakai *et al.* 2014 [7] within the phylum *Proteobacteria*.

Strain MWH-Nonnen-W8red^T 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-W8red^T was obtained from Lake Nonnenmattweiher located (geographic coordinates 47.795299°N and 7.798552°E) in the Black Forest Mountains (Schwarzwald), Germany, at an altitude 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-W8red^T 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 at room temperature (about 23 °C) a diameter of 5 mm after 18 days of incubation. No pronounced subsequent increase of colony diameter was observed. In liquid NSY medium (3 g/L, pH 7.2), the strain grew at 20 °C with a rate of 0.11 ± 0.003 h^{-1} (average and SD of three parallels) equalling a generation time of 6.5 h and reached a maximum OD_{575nm} of about 0.38. In the logarithmic 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 L^{-1} yeast extract, 0.1 g L^{-1} K_2HPO_4, 2.0 g L^{-1} agar) swarming colonies reached a diameter of 30 mm within three days. Upon storage at about 23° C for three 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 *Streptomyces* species (*Actinobacteria*). In order to exclude the possibility that slowly growing streptomycetes were co-isolated with strain MWH-Nonnen-W8red^T, the very cultures and spots from which the figures were taken, were inoculated to media typically used for streptomycetes (DSMZ media 65 (GYM Streptomyces Medium) and 987 (ISP2 Medium)), were subjected to Gram staining (Suppl. Mat. Fig. S1), and its 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 rDNA 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-W8red^T.

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. tunisiensuis* and *B. bacteriovorus* were not as densely packed as in strain MWH-Nonnen-W8red^T and in 'S. cienkowskii'.

The 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 OD at 575 nm 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 to the OD obtained without test substrate (i.e. in 0.3 g L^{-1} NSY medium) were scored after 10 days of growth as no utilization (-), weak utilization (w) and good utilization (+), respectively. The strain utilized D-mannose, D-glucose, L-proline, L-glutamate, and L-alanine. Three other tested substances were only weakly assimilated (Table 1). These assimilation experiments recorded growth of the strain based on optical density measurements. Additional substrate utilization

tests were performed with BIOLOG GN2 MicroPlates (BIOLOG, Hayward CA, USA), which detects utilization of substrates as electron donor 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 to inoculation in a 0.17% NaCl solution (the BIOLOG manual even suggests a 0.85% NaCl solution). The plates were read after 48 h incubation at 28°C. A threshold of 100 counts was set to evaluate a response as positive. The highest observed count was 232. These tests suggested that the strain is able to use alpha-D-glucose, alpha-ketobutyric acid, alpha-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 tested strain are in partial contradiction regarding utilization of some substances. In addition to experiments by these two methods, substrate utilization tests with API20NE strips (bioMérieux, Lyon, France) were performed according to the recommendations by the manufacturer. Interestingly, no substrate utilization nor any enzymatic reactions were detected with the API20NE strips.

Enzymatic activities of strain MWH-Nonnen-W8red^{T} were tested by using API Zym strips (bioMérieux, Lyon, France) 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-W8red^{T} 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 of growth, and salinity tolerance were not 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 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 analyzed after growth at 28°C on R2A and on NSY agar, respectively, by using an Agilent Technologies 6890N instrument and the Microbial Identification System (MIDI) Sherlock version 6.1 (results were evaluated against the TSBA 40 peak-naming table database) as described by Sasser [14]. Main compounds were iso-$C_{15:0}$, $C_{16:0}$, feature 3 including $C_{16:1}$ ω7c, anteiso-$C_{15:0}$, and $C_{17:0}$, however the composition differed between biomass grown on the two different media (Suppl. Mat. Table S4). A high number of 3-hydroxylated fatty acids were noticeable. In general, the fatty acid composition of strain MWH-Nonnen-W8red^T differed significantly from that given for *Oligoflexus tunisiensis* [7], in which $C_{16:1}$ ω5c and $C_{16:0}$ constituted 93 % of the detected cellular fatty acids.

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

The genome of strain MWH-Nonnen-W8red^T 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 obtained genome sequence has a length of 3.51 Mbp and a G+C content of 32.63 mol% and is characterized by a coverage of about 30x (Table 2). The resulting genome sequence was annotated using the 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-W8red^{T} 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 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 chromosome 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-W8red^T is quite similar to *Bdellovibrio bacteriovorus* HD 100^T and *Halobacteriovorax marinus* SJ^T, but the latter two lack plasmid-related type IV systems. By contrast, *Myxococcus* strains usually encode both a type III and a type VI secretion system. See below for the phylogenetic relationships of the mentioned taxa to strain MWH-Nonnen-W8red^T.

Relating to the below suggested phylogenetic relationships between strain MWH-Nonnen-W8red^T, *Oligoflexus* and the order *Bdellovibrionales*, it is interesting that the former two organisms do not possess the majority of the 59 genes present in all genome-sequenced members of the *Bdellovibrionales* but lacking in other previously investigated bacteria [21]. For instance, BLASTp searches resulted in the genome of strain MWH-Nonnen-W8red^T only in nine of the 59 query protein sequences in hits, 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-W8red^T lacks homologues of the *hit* locus, which is a conserved region in *Bdellovibrio* and *Halobacteriovorax* genomes known to encode functions involved in the predatory lifestyle of these bacteria [22].

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

231 Genome comparisons based on average nucleotide identity (ANI) analyses [23] of the MWH-
232Nonnen-W8red^T genome with the closest related type strains available (see below), i.e. with
233*Oligoflexus tunisiensis* Shr3^T [24], *Bdellovibrio* spp. [25, 26], *Halobacteriovorax marinus* SJ^T [22],
234and 'Bacteriovorax' spp. [21], resulted in quite low values of 66-69% ANI, which suggests only
235distant phylogenetic relationship between strain MWH-Nonnen-W8red^T and these taxa. In all three
236comparisons, these results obtained by using the IMG system [20] are based on alignment fractions of
237only about 1-2% of the genome sequences. Two way average amino acid identity (AAI) values
238calculated with the AAI calculator [27] for those genomes resulted in AAI values of about 35-38%,
239respectively. These results are based on alignments of >40% of the proteins encoded by the genome of
240MWH-Nonnen-W8red^T. ANI and AAI results both suggest only distant phylogenetic relationships of
241strain MWH-Nonnen-W8red^T to the compared taxa. It should also be mentioned that strain MWH-
242Nonnen-W8red^T and the reference taxon with the most similar 16S rRNA gene sequence, i.e.
243*Vulgatibacter incomptus* DSM 27710^T, even share an ANI value of 79.6% but the alignment fraction is
244less than 0.1% of the genome sequences.

245 The G+C value of the MWH-Nonnen-W8red^T genome of 32.6 mol% is exceptionally low for a
246proteobacterium with a genome size of > 3 Mbp (Suppl. Mat. Fig. S4). Among the 14,351 genomes of
247cultured *Proteobacteria* (environmental genomes were excluded) available in the IMG system [20] at
248the time of analysis (June 2016) characterized by genome sizes ≥ 3 Mbp were only 11 genomes found
249with G+C contents less than 35% (Fig. 2). Among these 11 taxa, no member of the current order
250*Bdellovibrionales* or the current class *Oligoflexia* are found. Interestingly, six genomes currently
251classified by the IMG system as 'Bacteriovorax' strains (including *Halobacteriovorax marinus* SJ^T),
252which should be considered as members of the below proposed new order *Bacteriovoracales* ord. nov.,
253possess G+C values in the range of 35-40%. However, the G+C values of the genomes of other
254members of the current order *Bdellovibrionales*, e.g. of *Bdellovibrio* spp., as well as of *Oligoflexus*
255*tunisiensis* Shr3^T, are higher than 40%. Thus, a G+C content of less than 40% is no common feature of
256the below proposed revised class *Oligoflexia*. Genomes with sizes smaller than 3 Mbp were excluded
257from these analyses, because genomes shaped by reductive genome evolution usually possess reduced

G+C values. However, of the 3002 proteobacterial genomes with sizes of less than 3 Mbp, 75% possess G+C values higher than the value of MWH-Nonnen-W8red^T.

We searched for other exceptional genomic or genetic traits of strain MWH-Nonnen-W8red^T. 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 *Proteobacteria* [28]. A systematic screening of 6212 proteobacterial genomes available in the IMG system revealed that strain MWH-Nonnen-W8red^T is quite exceptional in this trait. For this screening all genomes assigned to the classes *Alpha-*, *Beta-*, *Delta-*, *Epsilon-*, 'Zetaproteobacteria', *Acidithiobacillia* and *Oligoflexia*, respectively, were considered but due to the large number of genomes assigned to the *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 *Halobacteriovorax marinus* SJ^T. By contrast, the signature sequence was found among all other genome-sequenced strains currently classified as *Bdellovibrionales*, as well as in *Oligoflexus tunisiensis* Shr3^T. Twenty-two of the 34 genomes with substitutions in the signature sequence are currently classified as *Deltaproteobacteria*, which also includes the obviously misclassified *Vampirovibrio chlorellavorus* [29].

Furthermore, we tested if those taxa currently classified as *Deltaproteobacteria*, but proposed by us to be assigned to the class *Oligoflexia* (see below), could be distinguished from the other *Deltaproteobacteria* by the copy number of the ribosomal protein S1 gene. Karlin et al. [30] suggested that deltaproteobacterial 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 *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 investigated *Deltaproteobacteria* 46.4% encoded one, 53.0% encoded two, and 0.6% encoded three genes annotated as ribosomal protein S1 genes (COG 0539). Strain MWH-Nonnen-W8red^T, as well as none of the genomes currently representing the order *Bdellovibrionales* encoded two copies of the gene, however, *Oligoflexus tunisiensis* Shr3^{T}encodes two non-identical genes with this annotation. Obviously, the copy number of the ribosomal protein S1 gene is not a homogenous feature among bacteria currently classified as *Deltaproteobacteria* and is also not suitable for distinguishing those from other *Proteobacteria*.

For gaining a first hint on the phylogeny of strain MWH-Nonnen-W8red^T 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 most similar genes belong to various classes of *Proteobacteria*. Surprisingly, no type strains sharing a 16S rRNA gene similarity higher 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 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 by Williams and Kelly [31]. We selected a set of genome-sequenced reference strains representing the whole phylogenetic widths 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 *Oligoflexus tunisiensis* Shr3^T [24], which represents the latest described class of *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 (Integrated Microbial Genomes/Expert Review) system [20]. Protein families were identified by their COG (Clusters of Orthologous Groups) classification and families represented by more than one similar gene from the same genome in its COG category were usually rejected. Finally, the set of reference strains consisted of 84 strains (basic reference set, Suppl. Mat. Table S2) and the set of protein families consisted of 74 COGs (Suppl. Mat. Table S1). 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; Suppl. Mat. Tables S2 and S3).

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 average 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 construction 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-W8red^T consistently in a branch formed by *Bdellovibrio* spp., *Halobacteriovorax marinus* and *Oligoflexus tunisiensis* (Fig. 3). These trees confirm the status of *Oligoflexus tunisiensis* Shr3^T as the type of an own class of *Proteobacteria* [7]. However, these trees also suggest that the included representatives of

the *Bdellovibrionales* are, in contrast to their current classification, not affiliated with the class *Deltaproteobacteria*. In order to test the phylogenetic position of the *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 *Oligoflexus*, MWH-Nonnen-W8red^{T}, and members of the order *Bdellovibrionales* (Suppl. Mat. Fig. S3) except *Vampirovibrio chlorellavorus* [37, 38]. The reconstructed phylogenetic position of *V. chlorellavorus* confirms that this strain does neither belong to the order *Bdellovibrionales* nor to the phylum *Proteobacteria*, but is affiliated with the *Candidatus* phylum Melainabacteria [39]. This candidatus phylum represents a sibling phylum to the *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 *Oligoflexus*, MWH-Nonnen-W8red^{T}, and the order *Bdellovibrionales* within the class *Deltaproteobacteria* (Suppl. Mat. Fig. S3). Importantly, the affiliation of this lineage with the *Deltaproteobacteria* lacks any bootstrap support, while the separate phylogenetic positioning suggested by the previous multi-protein tree (Fig. 3) and 16S rRNA trees ([7], Suppl. Mat. 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 *Alphaproteobacteria* and the major branch of the *Deltaproteobacteria* but the extension of the taxon set shifted the position between the *Deltaproteobacteria* and the *Epsilonproteobacteria* (Suppl. Mat. Fig. S3).

The results of the performed phylogenetic analyses 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 *Proteobacteria* [31, 41-43] (Fig. 3; Suppl. Mat. Fig. S3). *Epsilonproteobacteria* and the deltaproteobacterial order *Desulfurellales* appear to be more distantly

related to the major part of the *Proteobacteria* than the phylum *Acidobacteria* ([31], Fig. 3). Besides the currently single species class *Oligoflexia*, which cannot be evaluated for monophyly, all other proteobacterial classes but *Deltaproteobacteria* appear to be monophyletic clades. Interestingly, the polyphyletic nature of the *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 *Proteobacteria* in its phylogenetic breath. 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-W8red^{T} proposed below requires a revision of the classification of the order *Bdellovibrionales*. Furthermore a reclassification of the species *V. 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 the 'Melainabacteria' is available yet, a revision of the classification of *V. chlorellavorus* has to be postponed until a member of this clade can be cultivated.

Does MWH-Nonnen-W8red^{T} represent 'Spirobacillus cienkowskii'? Strain MWH-Nonnen-W8red^{T} is regarding cell morphology, morphological variability and pigmentation very similar to 'Spirobacillus cienkowskii' characterized by Metchnikoff in 1889 [1]. Metchnikoff observed and described the life cycle of 'S. cienkowskii' in infected *Daphnia*. He reported various morphological forms, including rods, spirillae, and filaments. Appearance of such 'S. cienkowskii' morphotypes in infected *Daphnia* spp. was confirmed by Rodrigues and colleagues [2]. By using fluorescent *in situ* hybridization (FISH) probes specific for 'S. cienkowskii' they demonstrated that all these morphotypes belong to this taxon. We observed the same morphotypes in cultures of MWH-Nonnen-W8red^{T} including the unusual densely coiled spirals (compare Fig. 1 and Fig 2D in [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 the type strain of *Oligoflexus tunisiensis* [7, 10], which is much more distantly related to strain MWH-Nonnen-W8red^{T} as compared to 'S. 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 'S. cienkowskii' [45] and the genome of MWH-Nonnen-W8red^T encodes genes putatively enabling this organism to synthesise at least the red pigmented carotenoid lycopene (Suppl. Mat. Table S5). These phenotypical similarities between strain MWH-Nonnen-W8red^T and 'S. cienkowskii' are contrasted by differences in 16S rRNA and *gyrB* sequence. The 16S rRNA sequence of 'S. cienkowskii' determined by Rodrigues et al. [2] and the sequence of MWH-Nonnen-W8red^T share a similarity of only 95.9% (57 bp different), and importantly, are also distinct by five nucleotide insertions at two sites of the 'S. 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 G+C contents of about 36%. Interestingly, Rodrigues and colleagues found no differences in the 16S rRNA genes across a couple of European 'S. cienkowskii' populations but a sequences 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 'S. cienkowskii' populations across host species and continents makes it unlikely that MWH-Nonnen-W8red^T represents an 'S. cienkowskii'-like pathogen of *Daphnia* spp.

Other 16S rRNA sequences sharing similarities > 96% with the gene of strain MWH-Nonnen-W8red^T (Suppl. Mat. 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 with 'S. cienkowskii' 16S rRNA sequence similarities ≥ 96% do not represent obligate pathogens of *Daphnia* spp.

Two experiments were performed in order to test if MWH-Nonnen-W8red^T is able to infect *Daphnia* cf. *pulex*. The species *D. pulex* was reported to be susceptible to infections by 'S. cienkowskii' [2]. In a first experiment daphnids were challenged with 1.5×10^6 MWH-Nonnen-W8red^T cells mL^{-1}. Besides the added bacteria, the daphnids also received algae (*Cryptomonas* sp. 26.80) as food. The batch cultures (40 ml, six replicates) containing the daphnids were fed every 2-3

421days with a mixture containing algae and the tested bacteria. Throughout the experiment, which lasted
422for 23 days, the added food cocktail contained 100 times more bacterial carbon (MWH-Nonnen-
423W8red^{T}) than algal carbon (*Cryptomonas* sp.). Two controls were included in the experiment, both
424with six replicates (40 mL each). The first control (*Cryptomonas* only) received no bacteria but the
425same amount of algal carbon as the test treatments. The second control received instead MWH-
426Nonnen-W8red^{T} the terrestrial bacterium *Cupriavidus basilensis* DSM 11853^{T} [46] and the algal food.
427The total amount of carbon, as well as the carbon ratio of algae to bacteria (1:100) was identical in the
428two treatments receiving bacteria. The total number of daphnids and the number of off springs were
429counted during the experiment at 18 days. Special attention was paid to the appearance of red coloured
430daphnids and dead daphnids. Interestingly, the daphnids grew better in the control treatment without
431added bacteria, however, in the treatment, which received strain MWH-Nonnen-W8red^{T} the daphnids
432grew better than in the treatment with *C. basilensis* DSM 11853^{T} (Suppl. Mat. Fig. S7). Red pigmented
433daphnids or other hints on a bacterial infection were not observed in any replicate of all three
434treatments. A second experiment challenging daphnids with higher concentrations of MWH-Nonnen-
435W8red^{T} cells was conducted in order to test if infections occur at higher doses. Four parallel treatments
436receiving 3, 6, 9, and 12 x 10^{6} MWH-Nonnen-W8red^{T} cells mL^{-1} were established. All treatments
437received the same amount of algal food and were fed in the same way during the experiment. Again,
438infected daphnids were not observed in any of the four treatments.

439 In general, the two experiments did not result in any hint on a pathogenic potential of strain
440MWH-Nonnen-W8red^{T} regarding *Daphnia* cf. *pulex*, however, we cannot really exclude that the strain
441is able to infect daphnids if an appropriate host or appropriate infection conditions would be given. We
442note that a pathogenic potential of strain MWH-Nonnen-W8red^{T} could not be demonstrated so far.

443

444**Proposal of the new species *Silvanigrella aquatica* gen. nov., sp. nov. and required taxonomic**
445**revisions**

446 The large phylogenetic distance of strain MWH-Nonnen-W8red^{T} to any described species does
447not leave any doubt that this strain represents a new species. According to the obtained phylogenetic

trees, *Oligoflexus tunisiensis* and members of the order *Bdellovibrionales* represent the closest related described species. We propose to establish for the investigated strain the new 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 'S. cienkowskii' is too superficial to provide hints if strain MWH-Nonnen-W8red^T and those pathogens of daphnids should be placed in the same genus. Because of lack of evidence for pathogenicity in strain MWH-Nonnen-W8red^T and because of the inappropriateness of the name "bacillus" for a proteobacterium, we refrain from proposing 'Spirobacillus' as genus name for the new strain.

The 16S rRNA sequence similarity value of less than 82% between *Oligoflexus tunisiensis* Shr^T, the sole type strain in the class, and strain MWH-Nonnen-W8red^T, 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 *Silvanigrellales* ord. nov. of the class *Oligoflexia*. Furthermore, the multi-protein (Fig. 3) and the 16S rRNA 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 *Bdellovibrionales* within the *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 *Oligoflexus*, and the multi-protein tree presented here (Fig. 3) differ in bootstrap support for the class *Deltaproteobacteria*. While the tree lacking *Oligoflexus*, integrated *Bdellovibrio* in the *Deltaproteobacteria* but lacked bootstrap support for this class (39%), our tree excludes the *Bdellovibrionales* from the *Deltaproteobacteria* and supports the remaining class with high bootstrap supports in trees calculated with two out of three algorithms (Fig. 3, Suppl. Mat. Fig. S3). Only the NJ algorithm did not result in a sufficient bootstrap support. Based on phylogenetic analyses of multi-protein alignments, the transfer of the order *Bdellovibrionales* [49] from the class *Deltaproteobacteria* [50] to the class *Oligoflexia [7]* is proposed.

474 According to phylogeny and because none of the type species of the genera *Bacteriovorax*,
475*Peredibacter* and *Halobacteriovorax* share 16S rRNA sequence similarities of more than 82% with the
476type species of *Bdellovibrio* we propose to establish the new order *Bacteriovoracales* ord. nov. for
477those three genera (Fig. 4). Finally, based on phylogenetic analyses of 16S rRNA genes (Fig. 4), the
478transfer of the family *Pseudobacteriovoracaceae* McCauley *et al.* 2015 [44] from the order
479*Bdellovibrionales* Garrity *et al.* 2006 [51] to the order *Oligoflexiales* Nakai *et al*. 2014 [7] is proposed.
480The description of the family remains as given by McCauley *et al.* (2015) [44].

481

482**Description of *Silvanigrella* gen nov.**

483 *Silvanigrella* (Sil.va.ni.grel'la. N.L. fem. dim. n. *Silvanigrella* named after Silva nigra the
484Latin geographic name of the Schwarzwald (Black Forest) mountains located in the South-West of
485Germany).

486 The description of the genus is based on the polyphasic characterization of the type strain of
487the sole species proposed to be affiliated currently with this genus. Features probably characterizing
488other strains affiliated with this genus are as follows: Gram-negative, pleomorphic cell morphology,
489aerobic chemoorganoheterophs, red pigmentation.

490 The genus is a member of the class *Oligoflexia* [7] of the phylum *Proteobacteria [47]*. The
491type species is *Silvanigrella aquatica* sp. nov.

492

493**Description of *Silvanigrella aquatica* sp. nov.**

494*Silvanigrella aquatica* (a.qua'ti.ca. L. fem. adj. *aquatica* living, growing, or found in the water,
495aquatic).

496 The type strain is MWH-Nonnen-W8red^T (DSM 23856^T =CCUG 58639^T), which is the only
497strain investigated so far. Apart from the characters given for the genus, the species is characterized as
498follows: catalase weakly 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 chemoorganoheterotroph, anaerobic growth was neither observed on standard NSY medium nor 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 was quite weak. Assimilates D-mannose, D-glucose, L-proline, L-glutamate, and L-alanine. Weak assimilation of acetate, fumarate and glycine. No assimilation of glyoxylate, glycolate, propionate, oxaloacetate, malonate, lactate, citrate, D-xylose, D-fucose, D-sorbitole, L-methionine, and betaine. Main fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, feature 3 including $C_{16:1}$ ω7c and iso-$C_{15:0}$ 2-OH, $C_{16:0}$, $C_{17:1}$ ω8c and $C_{17:0}$. Main polar lipids are phosphatidylethanolamine and phosphatidylglycerol. Contains unidentified quinones, known ubi- and menaquinones could not be detected.

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 G+C content of 32.6 mol%. The genome sequence was deposited in DDBJ/EMBL/GenBank under the Accession Numbers CP017834 - CP017838.

Description of *Silvanigrellaceae* fam. nov.

Silvanigrellaceae (Sil.va.ni.grel.la.ce'ae. 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'les. 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* fam. nov. and *Candidatus* Turabacter [52], as well as undescribed or not validly described taxa, which were predominantly found in freshwater systems. This includes strains isolated from the skin of an amphibian [3], the pathogen of water flea 'Spirobacillus cienkowskii' [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 Kip [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'les. 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 *Bacteriovoracaceae* Davidov and Jurkevitch 2004 [54] and *Halobacteriovoraceae* Koval *et al.* 2015 [55]. The description of the order is based on the descriptions of the included 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.*, 2005a

Bdellovibrionales (Bdel.lo.vib.ri.o.na'les. N.L. masc. n. *Bdellovibrio*, type genus of the order; suff. *-ales*, ending denoting an order; N.L. fem. pl. n. *Bdellovibrionales*, the order of the genus *Bdellovibrio*).

Includes solely the genera *Bdellovibrio* Stolp and Starr 1963 [57], *Micavibrio* Lambina *et al.* 1982 [58] and *Vampirivibrio* Gromov and Mamkayeva 1980 [38], i.e. members of the illegitimate family 'Bdellovibrionaceae' [59]. The description of the order *Bdellovibrionales* remains as given by Garrity *et al*. [49] except for the exclusion of the families *Bacteriovoracaceae*, *Halobacteriovoraceae*, and *Pseudobacteriovoracaceae*. The type genus is *Bdellovibrio* Stolp and Starr 1963 [57].

Emended description of the order *Oligoflexiales* Nakai *et al.*, 2014

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

Encompasses the families *Oligoflexiaceae* and *Pseudobacteriovoracaceae*. The description is based on the descriptions of the genera *Oligoflexus* [7] and *Pseudobacteriovorax* [44]. Gram negative, chemoorganoheterotrophs, 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; suff. -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 cienkowskii' (Accession number of the 16S rRNA gene EU220836) and related cultured

and uncultured bacteria (compare Fig. 3 in [7]). Includes the orders *Oligoflexiales*, *Bdellovibrionales*, *Bacteriovoracales* ord. nov., and *Silvanigrellales* ord. nov.. The type order is *Oligoflexiales*.

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 Greek alphabet; Gr. or L. n. *Proteus*, Greek god of the sea, capable of assuming many different shapes; N.L. n. *bacter,* a rod; suff. -*ia*, ending to denote a class; N.L. neut. pl. n. *Deltaproteobacteria*).

The description of the class *Deltaproteobacteria* remains as given by [50] with the exception that the order *Bdellovibrionales* with its current taxa 'Bdellovibrionaceae', *Bacteriovoracaeae*, *Halobacteriovoraceae* and *Pseudobacteriovoracaceae* are excluded from the classis.

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CONFLICTS OF INTEREST

The authors declare the absence of any conflict of interest.

ETHICAL STATEMENT

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

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751

752**Tables**

753

754**Table 1.** Traits that characterize strain MWH-Nonnen-W8red^{T}. The assimilation data represent results 755of growth experiments performed as described previously [12].

756-, negative; +, positive; w, weakly positiveX

757

Characteristic	**MWH-Nonnen-W8red^{T}**
Cell morphology	pleomorphic
Cell length of rods (µm)	3.6
Cell width of rods (µm)	0.6
Motility (soft agar)	+
Catalase/oxidase	w/-
Temperature range of growth (°C)	10(w) – 32(w)
NaCl tolerance (%NaCl, w/v)	0 - 1.0(w)
Anaerobic growth:	
NSY medium	**-**
NSY enriched with nitrate	**-**
Assimilation of:	
Glyoxylic acid	-
Glycolic acid	-
Acetic acid	w
Propionic acid	-
Oxaloacetic acid	-
Malonic acid	-
DL-Lactate	-
Fumaric acid	w
Citric acid	-
D-Xylose	-
D-Mannose	+
D-Glucose	+
D-Fucose	-
D-Sorbitol	-
Glycine	w
L-Proline	+
L-Glutamate	+
L-Alanine	+
L-Methionine	-
Betaine	-

758X

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761**Table 2.** Genome characteristics of strain MWH-Nonnen-W8red^{T}.

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Scaffold	Type	Accession Number	Size	No. Genes	GC (Mol%)
1	Chromosome	CP017834	3.34 Mbp	2896	33
2	Putative conjugative plasmid	CP017835	42.2 Kbp	48	30
3	Putative prophage	CP017837	41.8 Kbp	58	31
4	Putative prophage	CP017838	43.3 Kbp	58	36
5	Putative conjugative plasmid	CP017836	37.0 Kbp	42	29

763

764Mbp, mega base pairs; Kbp, kilo base pairs.

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Figure legends

Fig. 1. Scanning electron microscopy images. Most visible bacteria show a filamentous morphology when growing in soft agar while some bacteria form spirals (arrows in A) with no visible constrictions or separations along the spirals (B).

Fig. 2. Frequency distribution of G+C values of *Proteobacteria* genomes available in the Integrated Microbial Genomes (IMG) system characterized by genome sizes of ≥ 3 Mbp. Only four and eight (including MWH-Nonnen-W8red^T) genomes possess G+C values in the range of 25-30 and 30-35 mol %, respectively.

Fig. 3. RAxML tree calculated with an alignment of 74 protein sequences extracted from 85 genome sequences. Taxa affiliated with the phylum *Proteobacteria* are shown by using colour codes. All proteobacterial classes (including 'Zetaproteobacteria') but *Deltaproteobacteria* are shown by colour-coded fonts. Deltaproteobacterial orders are highlighted by colour-coded areas. Furthermore, the two representatives of the phylum *Acidobacteria* are highlighted by red fonts. Bootstrap values obtained by the RAxML, MrBayes and Neighbour-Joining method are depicted. The coloured dots indicate nodes supported by different treeing methods.

Fig. 4. Revised taxonomy of the class *Oligoflexia*. The shown Neighbour-Joining tree was calculated with almost complete 16S rRNA 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 outgroup (not shown). Bootstrap values obtained with the neighbour joining, the maximum likelihood and the maximum parsimony methods (1000, 100, 100 replications, respectively) are indicated. Note that this 16S rRNA tree differs regarding the position of the revised *Bdellovibrionales* from the calculated multi-protein trees (Fig. 4 and Suppl. Mat. Fig. S3), however the responsible node is only weakly supported in the 16S rRNA tree. The branching order of the calculated NJ and ML tree is identical.