1 Taxonomic study of nine new *Winogradskyella* species occurring in the shallow waters of
2 Helgoland Roads, North Sea. Proposal of *Winogradskyella schleiferi* sp. nov., *Winogradskyella*
3 *costae* sp. nov., *Winogradskyella helgolandensis* sp. nov., *Winogradskyella vidalii* sp. nov.,
4 *Winogradskyella forsetii* sp. nov., *Winogradskyella ludwigii* sp. nov., *Winogradskyella ursingii*
5 sp. nov., *Winogradskyella wichelsiae* sp. nov., and *Candidatus "Winogradskyella atlantica"* sp.
6 nov.

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Con formato: Español (España)

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Abstract:

Evaluation of bacterial succession with cultivation-dependent strategies during a spring phytoplankton bloom in the North Sea led to the isolation of 41 strains that affiliated with the genus *Winogradskyella*. Fifteen of the strains were selected for a taxonomic study after discarding clonal cultures. A thorough phylogenetic, genomic and phenotypic analysis of the isolates indicated that they represented eight new species that coexisted in North Sea waters. Molecular data revealed the existence of an as yet uncultivated novel species recurrently binned from the North Sea metagenomes. The metagenome-assembled genomes (MAGs) of this new *Winogradskyella* were used to classify it as a new *Candidatus* species. It represented a new example of the use of the tandem approach of whole cell mass spectrometry linked to 16S rRNA gene sequencing in order to facilitate the discovery of new taxa by high-throughput cultivation, which increases the probability of finding more than a single isolate for new species. In addition, we demonstrated the reasons for classifying MAGs representing recurrently retrieved heterotrophic species that evade cultivation even after an important high-throughput effort. The taxonomic study resulted in the classification of eight new species and one new *Candidatus* species of the genus *Winogradskyella* for which we propose the names *W. schleiferi* sp. nov., *W. costae* sp. nov., *W. helgolandensis* sp. nov., *W. vidalii* sp. nov., *W. forsetii* sp. nov., *W. ludwigii* sp. nov., *W. ursingii* sp. nov., *W. wichelsiae* sp. nov., and *Candidatus* "W. atlantica" sp. nov.

Key words: Bacteroidetes, polysaccharide utilization, genomics, metagenomics, Candidatus,

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Introduction:

Winogradskyella is a genus of the phylum *Bacteroidetes* classified within the family *Flavobacteriaceae* [5] with the type species *W. thalassocola* [37]. Currently, the genus consists of 34 classified species as listed in <https://lpsn.dsmz.de/genus/winogradskyella> [45], and has been emended on multiple occasions [4, 16, 38, 71]. The members of the genus have a very versatile phenotype, since they appear as non-sporulating rod or coccoid Gram-negative cells, which form colonies with diverse morphologies and yellow-, orange-, or salmon-colored pigmentation, but without flexirubins. Cells are either non-motile or motile by gliding, while some can form network-like structures; they are strictly aerobic or facultatively anaerobic and chemoorganotrophic with a NaCl range between 0.0-10% (w/v). Depending on the species, strains grow at a broad range of temperatures between 4 and 44 °C.

Members of *Winogradskyella* have been isolated from marine environments, including plankton (e.g. [18]) and sediments (e.g. [19]). Many strains have originated from samples associated with marine invertebrates, such as sponges (e.g. [56]), corals [13], mollusks (e.g. [41]), and echinoderms (e.g. [40]), while others came from marine vertebrates, such as tuna [62], algae (e.g. [22]) or inert surfaces of marine origin (e.g. [63]). *Winogradskyella* spp. have been reported to be polysaccharide degraders that have a very diverse arsenal of enzymes for dealing with complex and diverse macromolecules, especially polysaccharides. In addition, some species of the genus have been reported to have biotechnological relevance, for example, for their antifouling effect [7], or for the genetic engineering of their rhodopsins [9].

Cultivation-dependent strategies of bacterial succession during a spring phytoplankton bloom in the North Sea led to approximately 5,000 strains being isolated in pure culture from the shallow waters of Helgoland Roads, located in the North Sea German Bight [2]. From among the more than 365 species detected in the North Sea survey, 41 isolates were identified as members of the genus *Winogradskyella*, and they were selected for a genomic study (Alejandre-Colomo, unpublished results). 16S rRNA gene-based phylogenetic reconstruction affiliated the new strains with nine different clades, several of them representing new species due to their unique phylogenetic position and genetic divergence from their closest relatives. After dereplication using DNA fingerprinting techniques, the genomes of 15 representative strains were sequenced and compared, and eight of the pure culture clades and two metagenome-assembled genome (MAG) collections represented as yet unclassified genomospecies of *Winogradskyella*. The present study therefore represented another good example of the use of the tandem approach of cultivation - MALDI-TOF MS - 16S rRNA gene sequencing [64], which overcomes the lack of multiple strains in species descriptions [8, 53, 61]. The tandem approach consists of using whole cell biomass taken directly from fresh cultures for analysis using MALDI-TOF mass spectrometry, which provides simple profiles reflecting to a large extent the major ribosomal proteins [67]. Clustering based on similarity values between profiles allows the grouping of cultures into species or groups of closely related species. To identify each cluster, only a few representative members of each group need to have their 16S rRNA gene sequenced, and therefore identified. This process reduces costs significantly and therefore allows a significant increase in the number of strains tested, thus enhancing the chances of isolating multiple strains of the same taxon [67]. In this current study, we formally propose new specific names for eight new species of the genus

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Winogradskyella that have been isolated in a high-throughput cultivation survey [2], and the classification of a new *Candidatus* member of the genus that corresponded to the most abundantly retrieved genotype of the genus in German Bight waters using culture-independent methods, and that evaded cultivation.

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Materials and Methods:

Strains and cultivation media:

All strains used in this study were obtained using the high-throughput cultivation approach performed during the spring bloom of 2016 [2] at the 'Kabeltonne' station of the Helgoland Roads (54° 11' 17.88" N, 7° 54' 0" E). The subset of 15 strains, affiliated to the genus *Winogradskyella* and for which a genome study had already been performed (Alejandre-Colomo, unpublished results), was used for comparison with the type strains of all classified species of the genus (Table 1). For biomass production, MA (Marine Agar, Pronadisa, ref: 1217) was used to check colony morphology and pigmentation, as well as to test certain other traits on agar plates. Colony morphology was observed after 10 days incubation at 12 °C. Cell morphology was observed using differential interference contrast (DIC, Nomarski) microscopy (Zeiss Axio Imager) for cultures in the exponential growth phase in SW3% [50] that were fixed with 3.0% formaldehyde. Gliding motility was determined as described by [6]. The temperature growth range was measured on MA in the range 4 - 35 °C at intervals of 4, 12, 20, 25, 30 and 35 °C. The salinity range was tested on SW medium [50] supplemented with 0.05% yeast extract and 0.15% pyruvate with the salt concentration ranging between 1% and 10% NaCl in intervals of 1%. The pH growth range was determined on MA adjusted on autoclaved media to a range of pH 4.0–11.0 (with increments of 0.5 pH units) using NaOH or HCl 100 mM, and filter sterilized before inoculation. Hydrolysis of casein, starch, Tween 20 and Tween 80, and deoxyribonuclease (DNase) activity were tested according to the methods described by Cowan and Steel [10]. Agarase activity was verified as previously described [14]. Oxidase activity was determined by oxidation of swabs impregnated with oxidase reagent (N,N-Dimethyl-p-phenylenediamine dihydrochloride, 1.0 mg, Scharlab, ref: 06-120-050) according to the manufacturer's instructions. Catalase activity was tested by measuring the production of oxygen bubbles in 3% (v/v) aqueous hydrogen peroxide solution. Biochemical tests and enzyme activities were carried out by using API 20NE and API ZYM (BioMérieux) according to the manufacturer's instructions. The results were read daily over a 5-day incubation period at 25 °C and after incubation for 4 h at 37 °C, respectively.

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Metagenome-assembled genomes (MAGs):

MAGs from bacterioplankton (0.2 – 3.0 µm size fraction) at the Helgoland Roads sampling site have been published previously (with accession project number PRJEB28156; [28]). From these, 15 MAGs identified as *Winogradskyella* sp. were used from the four years 2010 (3 MAGs), 2011 (7 MAGs), 2012 (1 MAGs), and 2016 (4 MAGs). Full names as presented in PRJEB28156, and their corresponding abbreviated names used throughout this article, can be found in Supplementary Spreadsheet S1. Taxonomic parameters, annotation and genomic comparisons were determined as given in the section for genomic analyses.

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Phylogenetic reconstruction and genome analyses:

The genomic DNA and the sequence of the isolates had been determined as previously reported [66]. The genome sequences were deposited in the NCBI database under the codes JABFDI000000000; JABFDK000000000; CP053352; JABFHO000000000; JABFDF000000000; JABFDC000000000; JABFDD000000000; JABFDJ000000000; JABFDG000000000; JABFDE000000000; CP053350; CP053349; CP053348; CP053351 and JABFDH000000000. *W. undariae* strain CCUG 63832 was sequenced by the CCUG and deposited under the accession code JABTEL000000000. 16S rRNA gene sequences were extracted using barrnap v0.9 (see <https://github.com/tseemann/barrnap>). All available *Winogradskyella* 16S rRNA sequences from described species and sequences from the genomes were imported into the reference database LTP_SSU_132 and aligned using SINA v.1.3.1 (SILVA Incremental Alignment [46]) implemented in the ARB software package v6.0.6 [35]. Final alignments were manually improved following the reference alignment in ARB-editor. Complete sequences were used to reconstruct *de novo* trees using the neighbor-joining [55] and RAxML [59] algorithms with the bacteria conservational filter, using Jukes-Cantor and GTRGAMMA corrections, respectively. The partial sequence from MAG Bin_69_1 was added to the reference tree using the Parsimony tool. Phylogenetic reconstructions using the core genome were generated by aligning single-copy orthologous genes present in the complete genome dataset, and translated to proteins, using MUSCLE v3.8.31 [11]. Aligned proteins were then concatenated and used to reconstruct genealogies using the RAxML [59] and neighbor-joining [55] algorithms, as implemented in ARB v6.0.6 [35]. The average nucleotide identity (ANI) and the average amino acid identity (AAI) between all genomes were determined according to Konstantinidis and Tiedje [27] using the scripts available at <http://enve-omics.gatech.edu/> [49].

Fatty acid profiles:

Fatty acids were extracted from total cell lysates grown on MA after 48 h incubation at 28 °C, and fatty acid analysis was performed according to Kämpfer and Kroppenstedt [17] by the separation of fatty acid methyl esters using gas chromatography (5898A, Hewlett Packard). Peaks were automatically integrated and fatty acid names and percentages were determined with the Sherlock MIDI version 2.1 (TSBA version 4.1).

Results and Discussion:

New species of Winogradskyella using culture-dependent approaches.

From the high-throughput cultivation survey in Helgoland waters [2], 41 strains were identified belonging to the genus *Winogradskyella*, which affiliated with 10 distinct OPUs representing different species. In general, most of the OPUs were represented by several isolates, but in some cases their genomic fingerprints indicated that these were clonal strains, and therefore they were not included in the taxonomic study. Only two of the clades harbored two or more different strains (OPU05 and OPU07 with two and four strains, respectively). The 15 strains for which the genome was available were taxonomically evaluated by means of their 16S rRNA gene (Figure 1 [and Supplementary Spreadsheet 2](#)) and core genome (Figure 2) genealogies, and also from their genomic (Table 2) and phenotypic (Table 1 and Table 3) coherence as the basic premises to circumscribe species [53]. The 16S rRNA consensus phylogeny (Figure 1) showed that all strains formed independent clades within the lineage embracing the majority of the *Winogradskyella* species, except for OPU01 and OPU10, which affiliated with *W. undariae*, and

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OPU02, which affiliated with *W. pacifica*. The 16S rRNA sequence divergence of the six remaining OPU02s from their own closest relatives seemed to be large enough to be considered as new species. Five out of the ten OPU02s (OPU01 with 10 isolates, OPU04 with three isolates, OPU05 with seven isolates, OPU06 with two isolates, and OPU07 with 14 isolates) were represented by two or more strains [2], whereas the remaining OPU02s were singletons. OPU03 was represented by strain HL116 and it had a 16S rRNA gene identity of 97.8% with its closest relative type strain of *W. eximia*; OPU04 represented by Z3138 had a 16S rRNA gene identity of 98.8% with *W. undariae*; OPU05 represented by strains Z953 and Z354 had a 16S rRNA gene identity of 98.3-98.6% with *W. pacifica*; OPU06 represented by HL364 had a 16S rRNA gene identity of 98.5% with *W. sediminis*; OPU07 represented by strains HL857, HL6110, Ha767 and SW133 had a mean 16S rRNA gene identity of 96.7-96.8% with *W. litoriviva*; OPU08 represented by Z215 had a 16S rRNA gene identity of 97.9% with *W. profunda*; and OPU09 represented by HL4150 showed a 16S rRNA gene identity of 96.0% with *W. exilis*. The tree topology of the small ribosomal subunit was in good agreement with the tree generated by the concatenation of the core genes of the genus (Figure 2). Apart from some branching order that was not reproduced, especially for the position of *W. undariae*, the overall topology agreed.

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The whole genome analysis of all sequenced strains together with all available type strain genomes of the closest relative species reinforced the idea that most of the OPU02s represented novel species. ANI [48] and AAI [27] with the closest relative type strains were always <91.0% (Table 2), which was well below the species threshold [48]. The genus status embracing all genomes analyzed was guaranteed, since all but *W. aurantiaca* had AAI values >70.0%, which is a good threshold for this category [27]. Such a distance with the core species of the genus, as well as the precise genome analyses of the gene content (Alejandre-Colomo, unpublished results) did not justify splitting *Winogradskyella* into different genera.

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As a summary of the genomic traits relevant for taxonomic purposes, there was a similarity to all species of the genus in the G+C mol% content of their genomes, which ranged between 32.3% for strain Z738 (OPU10) to 35.0% for strain HL4150 (OPU09). However, this variation was well within the expected range for the whole genus (Table 1 and Table 2). The genome size of the isolates also fluctuated within that observed for the different *Winogradskyella* type strains. From our isolates, HL4150 (OPU09) had the smallest genome with 3.5 Mbp and SW133 (OPU07) the largest with 4.90 Mbp. The latter is to date the largest sequenced genome within the genus *Winogradskyella* (Table 2). From the genomic characteristics and the phylogenomic analyses, the isolates were hypothesized to represent different lifestyles (Alejandre-Colomo, unpublished results). On the one hand, the phylogenetic branch harboring most of the isolates (Figure 1), all with larger genomes (with an average of 4.15 Mbp $\pm$ 0.39; ranging from 3.38 Mbp for *W. epiphytica* CECT 7945^T to 4.87 Mbp for SW133), and larger sets and diversity of polysaccharide degradation genes (PUL genes) were hypothesized as probably being related to a surface-attached lifestyle, a fact that was reinforced by the low yields on isolation from pelagic waters, whereas a relevant representation in the metagenomes originated from the attached fraction. On the other hand, a secondary branch with most of the MAGs from the North Sea surveys [28], with a mean genome size of 2.60 Mbp $\pm$ 0.70 (ranging from 1.16 Mbp for Bin_46_1 to 3.52 Mbp for *W. tangerina* M1309^T) was hypothesized to have a free-living lifestyle. The OPU09 represented by HL4150 was the only one of our isolates that affiliated with this branch, together

with other species of planktonic origin (Figure 1), such as *W. aurantiaca* [58] and *W. aquimaris* [34]. However, the fact that other species of the same clade had been isolated as putatively associated with surfaces, such as *W. tangerina* and *W. aurantia* from sediments [60, 67], or *W. jejuensis* [22] and *W. algicola* both associated with a brown alga [33], and *W. poriferorum* associated with sponges [31], did not preclude the possibility that their lifestyle could have been planktonic and they were only embedded within the porewaters of the sediments or the marine water films of the wet biological material collected. In addition, OPU08 (represented by Z215) was the only isolate of *Winogradskyella* that encoded a rhodopsin, a characteristic that seems to be related to a pelagic lifestyle and that has a potential use in biotechnology [9]. In addition, the all-versus-all *Winogradskyella* genome pairwise analysis between genomes with a completeness higher than 95% yielded a total of 13,154 orthology groups (OGs; Supplementary Spreadsheet S2S3). All genomes shared 1,065 OGs (core-genome of *Winogradskyella*; Supplementary Spreadsheet S3S4) mainly encoding genes with central metabolism (179 OGs were assigned to uncharacterized proteins; Supplementary Spreadsheet S4). The auxiliary genome comprised 12,089 OGs, of which 4,115 belonged to genome specific OGs (Supplementary Spreadsheet S4S3). The pan-genome configuration indicated an open-genome trend (Supplementary Spreadsheet S5S5).

After 10 days on MA, all strains formed shiny viscous colonies 1-2.5 mm in diameter, with the exception of Z738 that formed very large colonies ranging from 1.0-1.7 cm (Supplementary Table S1). In general, four types of morphology could be observed (CS, RZ, IF, IR) (Table 1), ranging from entire and undulate edges to rhizoid. All colonies were pigmented, and their color ranged from orange (strains Z3138 and HL857), salmon (strains Z215), and yellow (strains R3187 and HL4150) to orange-yellow (the great majority). The cell shapes were also very diverse (Supplementary Table S1) and they varied in length. In general, cells were of bacillary or coccobacillary form, whereas, in some cases, growth occurred in the form of aggregates and some filamentous cells also appeared when grown on MA. All isolates of OPU07 and the single strain of OPU08 Z215 formed biofilms that adhered to the tube surface when growing on SW3%. In some cases, cells with spheroplast morphology (the isolates of OPU01 Ha325 and Z3185, all isolates of OPU07 and the single isolate of OPU10 Z738) could also be observed. To date, this morphology degeneration has not yet been reported for *Winogradskyella*, although a close relative member of the *Flavobacteriaceae*, the species *Flaviramulus basaltis*, generated non-viable spheroplasts in the stationary phase [12]. The viability of the spheroplasts was not studied further, but it was remarkable that these degenerated cells already appeared in the exponential phase, and only in the members of a single clade (Figure 1). All strains grew within a temperature range of 4 °C to 30 °C, and no growth was seen below or above this range. All strains required salt of at least 1% NaCl for growth, but the upper range varied depending on the strain. The highest tolerance was for strains R3187 (*W. pacifica*) and Z215 (OPU08) that had an upper range of 10% NaCl. All strains were positive for hydrolysis of Tween 20, starch, aesculin hydrolase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase. All strains were negative for hydrolysis of casein, tyrosine, cellulose and Tween 80, indole production, glucose fermentation, arginine dihydrolase, urease and assimilation of glucose, arabinose, mannose, mannitol, N-acetyl-glucosamine, maltose, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid, trypsin, α -galactosidase, β -glucuronidase, α -

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Comentado [CJR1]: Are clades interchangeable with groups, as depicted in Figure 1?

glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase, and α -fucosidase. The differential diagnostic traits between strains and the different classified members of the genus are shown in Table 1. In general, all the new specific profiles matched well with the heterogeneous phenotypic repertoire of the genus, which embraced 42 species using our new classifications. Each specific diagnostic profile is provided in the formal taxonomic descriptions in Table 4.

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The fatty acid profiles (Table 3) of the new isolates matched the general profile of the majority of representative members of the genus, exhibiting the branched iso-C_{15:1} G and iso-C_{15:0}, and the hydroxylated iso-C_{16:0} 3-OH as major components. However, certain specific differences could be found among the OPUs studied, such as, for example, the relative relevance of iso-C_{15:0} 3-OH for strain HL116 (OPU03); iso-C_{15:0} 3-OH and iso-C_{17:0} 3-OH for the single strains Z3138 (OPU04) and HL634 (OPU06); anteiso-C_{15:0} and iso-C_{15:0} 3-OH for both strains of OPU05; iso-C_{17:0} 3-OH for all four strains of OPU07; iso-C_{15:0} 3-OH for the single strain Z215 (OPU08); or summed feature 3 of strain HL4150 (OPU09), and iso-C_{15:0} 3-OH, anteiso-C_{15:0} and iso-C_{17:0} 3-OH for strain Z738 (OPU10).

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A phenotypic property that clearly identified each of the new species as unique was difficult to find within the framework of the global set of 42 *Winogradskyella* species (considering that here the genus diversity was expanded with eight additional species). In some cases, such as OPU07, the presence of β -galactosidase, or in OPU04 and OPU06 the denitrification capability, could work well as diagnostic properties. Nevertheless, considering that most of the species of the genus have been classified by only using single strains, the intraspecific variability could be considerable (see for example the differences between *W. undariae* and *W. pacifica*; Table 1). Furthermore, there is an important laboratory to laboratory discrepancy in the results using the same strain and standardized commercial tests (highlighted in Table 1). Therefore, we think that the best diagnosis for identifying an organism as a member of one of these species is matching genomic coherence using the ANI values and the interspecific ANI gap [53].

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Altogether, the genomic and phenotypic parameters measured in this current study indicated that the new clades of *Winogradskyella* isolated from the Helgoland Roads represented a total of eight new species for which we propose the names *W. schleiferi* sp. nov. for OPU03, which is named after Prof. K.H. Schleifer for his contributions to prokaryotic taxonomy; *W. costae* sp. nov. for OPU04 in memoriam of Prof. M. da Costa for his contributions to bacterial and archaeal taxonomy; *W. helgolandensis* sp. nov. for OPU05 that was comprised of two strains, one of which detected MAGs in metagenome surveys (unpublished data); *W. vidalii* sp. nov. for OPU06 named after Prof. R. Vidal for his contributions to microbiology; *W. forsetii* sp. nov. for OPU07 that was comprised of four strains, named after the god of justice and reconciliation in Scandinavian mythology; *W. ludwigii* sp. nov. for OPU08 named after Dr. W. Ludwig for his contributions to prokaryotic molecular phylogenies; *W. ursingii* sp. nov. for OPU09 in memoriam of Prof. J. Ursing for his contributions to bacterial taxonomy; and *W. wichelsiae* sp. nov. for OPU10 named after Dr. A. Wichels for her scientific dedication to the Helgoland Research Station. The protologues describing the diagnostic traits, the designated type material and the metadata accompanying the description are given in Table 4.

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New species of Winogradskyella using culture-independent approaches.

Current metagenomic approaches can allow retrieval of over 90% of microorganisms, often the almost full genome sequence, thus avoiding the cultivation step by using the bioinformatic tools that bin contigs into metagenome-assembled genomes (MAGs). Depending on the abundance of organisms, MAGs often represent a mosaic of genes for the different ecotypes of the same species (i.e. the pangenome) coexisting in the environment [25]. The genome information obtained for each single major population has at least the same taxonomic value as that obtained by cultured organisms [24]. However, to name a taxon validly, the International Code of Nomenclature for Prokaryotes (ICNP; [44]) requires the deposit of a pure culture of a designated type strain for each description in two independent and international culture collections in order to preserve a living specimen and permit its availability to the scientific community. This requirement clearly impedes the formal naming of non-cultivated taxa, and has been challenged by the request to implement, as an alternative type material, the DNA sequence of genomes [68, 69]. Unfortunately, among the members of the scientific community of microbial taxonomists, there is still a certain reluctance to recognize that the current taxonomic schema has been mostly constructed primarily by whole genome comparisons modulated by 16S rRNA phylogenetic reconstruction approaches [53]. It has also been argued that allowing DNA to become the alternative type material would severely discourage the isolation of microorganisms in pure culture [54]. Therefore, and despite the fact that we believe the advantages of this radical change in the ICNP outweigh the concerns of the community [26], the International Committee for Systematics of Prokaryotes (ICSP) has not only rejected the proposals to expand the nature of the type material [69], but also that the *Candidatus* names should be granted priority [70]. The lack of success in the modernization of the ICNP has left the community of molecular microbial ecologists with the need to find a solution themselves, as has already been predicted [25], which most probably will be to create an independent code and regulating body in order to classify and name single-amplified genomes (SAGs) and MAGs [36]. As already undertaken in the past [65], we believe that MAGs can be taxonomically classified, and that the best approach is to record them as candidate taxa.

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In this *Winogradskyella* ecogenomic study (Alejandre-Colomo, unpublished results), 15 highly similar monophyletic MAGs (OPU11; Figure 2) were included with ANI values ranging from 99.7% to 99.95% (Supplementary Table S2). These MAGs were retrieved from 15 metagenomes obtained in four distinct years (three in 2010, seven in 2011; one in 2012 and four in 2016) from the same sample site, the Helgoland Roads station (with project accession number PRJEB28156; [28]). Only one MAG, Bin_69_1 (with accession number 20110512_Bin_69_1) binned a partial 16S rRNA gene sequence of 405 nucleotides (NCBI accession number MT577846), whose closest relative sequences with 100% identity were a clone from a sediment sample from the coast of Galicia (seq. JQ579718; [1]) and a clone from a microbiome associated with the toxigenic diatom *Pseudo-nitzschia multiseriata* (seq. HM140668; [15]). Interestingly, we also found a 99.8% identical 16S rRNA gene sequence with a clone from exactly the same location, also from a planktonic sample, that was taken in August 2000 (seq. AM279207; [3]), indicating the prevalence of members of this species over a time span of at least 20 years. The cultivated species closest relatives were all members of the clade (Figure 1) comprising *W. jejuensis* (with 97.8% identity), *W. haliclona* (94.3% identity), *W. wandonensis* (95.5% identity), and *W. litorisediminidis* (94.8% identity). Bin_69_1, with 97.1% completeness, was taken as the

reference MAG because this was the only one with a 16S partial gene sequence. Some others had a ~99% completeness (Supplementary Table S2), and therefore we considered the pangenome of this group (i.e. the complete set of genes as a sum of all MAGs) as the reference to infer metabolism. The collection encoded a small genome with a size of ~2.3 Mbp, which was in accordance with the hypothesized free-living lifestyle (Alejandre-Colomo, unpublished results). All MAGs showed a GC mol% content of $33 \pm 0.038\%$, and ANI and AAI of >99% between them (Supplementary Table S2). North Sea MAGs shared 1,765 orthologous genes with the genome of the type strain of the closest species *W. jejuensis* DSM 25330^T, with which they shared between 80.9% and 81.8% AAI and 78.8 and 79.0% ANI. From the orthologous genes, 459 were uncharacterized proteins. *W. aurantia*, *W. aurantiaca* and *W. tangerina* species shared 1,841, 1,829 and 1,896 OGs with Bin_69_1, respectively. The MAG encoded a total of 2,261 proteins, of which 186 OGs were exclusive to this genome, and 107 of them belonged to uncharacterized proteins. According to KEGG metabolic reconstruction, the genomic group encoded for complete glycolysis, gluconeogenesis, pyruvate oxidation, the TCA cycle (Krebs cycle) and the pentose phosphate pathway, all of which belonged to central carbohydrate metabolism. In comparison with *W. jejuensis* (DSM 25330^T; FQWS00000000), they lacked the pathway for glycogen biosynthesis and the glyoxylate cycle. Both genomic groups encoded the same genes related to fatty acids, nucleotide and amino acid metabolism.

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No special trait could be found that would really be diagnostic for their phenotype (Supplementary Spreadsheet S6), but altogether the organisms represented by these MAGs should be aerobic heterotrophs, most probably with a planktonic free-living lifestyle. This latter hypothesis is based on the reduced genome size, the presence of a proteorhodopsin gene typical of planktonic bacteria, and the fact that the MAGs were assembled from the 0.2-3 µm size fraction, which is depleted of particles and phytoplankton and enriches for planktonic bacteria. Another of the conspicuous characters that pointed to a free-living style was the presence of a reduced set of only two polysaccharide utilization loci (PULs). These were for β-glucan and the GH92-GH20-GH2-CE4 PULs (Alejandre-Colomo, unpublished results) for which substrate specificity is difficult to predict. Conspicuously, this genotype lacked a PUL for α-glucan, which is widely represented in the genomes of culturable *Winogradskyella*. The organism would be most likely motile by gliding as all genes related to this (Supplementary Spreadsheet S6S7) that make up the conserved *Bacteroidetes* core gliding-essential genes [30] could be detected. In comparison with the phenotypic traits used to generate diagnostic Table 1, the organisms encoded, and therefore would probably be positive for, oxidase, β-galactosidase, trypsin, N-acetyl-β-glucosaminidase, DNA hydrolysis, and probably starch degradation, since they encoded the GH13 family CAZymes. Nevertheless, these genes could not be detected and, therefore, they were considered negative for the traits related to gelatin or casein hydrolysis, nitrate reductase or the genes for the complete denitrification pathway, agarase, arginine dihydrolase, α-chymotrypsin, β-glucosidase or glucose fermentation.

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According to the read recruitments in their corresponding metagenomes, this as yet uncultivated genotype was always present in low abundance, reaching in one case a maximum relative abundance of 1.46%. However, it was repeatedly retrieved in several metagenomes from 2010 to 2012, and throughout the spring bloom in 2016 at Helgoland Roads. We believe that the species related to Bin_69_1 must also be adapted to cold waters as, from among the

47 metagenomes from the North Sea surveys [28, 47] and the 470 metagenomes from the Tara Oceans survey, it could only be detected in metagenomes from temperate waters in the Atlantic Ocean (Alejandro-Colomo, unpublished results). Altogether, we believe that this recurrently retrieved genotype, that showed high completion (97.1% to 99.0%) in its genome composition and low contamination (0.0%-2.9%), represented a single species of probably free-living *Winogradskyella*, adapted to cold waters, that could have an important role in the degradation of complex carbohydrates dissolved in planktonic waters. Therefore, we further believe that given the prevalence, completeness of the genome information and uniqueness within the genus, it deserves to be classified as a new candidate taxon, for which we propose *Candidatus* “*Winogradskyella atlantica*” sp. nov. The formal description of this new taxon is given in Table 4 together with the descriptions of the eight cultivated species.

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As mentioned in the introduction, this study represents a good example of where the tandem approach of cultivation – MALDI-TOF MS – 16S rRNA gene sequencing [67] overcomes the lack of multiple strains in species descriptions [8, 53, 61], and also comprises a relatively cost-effective effort for the discovery of taxonomic novelty. However, it is also a good illustration of the need for a stable nomenclature for uncultured taxa that are currently retrieved using cultivation-independent methods and cannot be obtained in pure culture. The members of *Candidatus* “*Winogradskyella atlantica*” must have been present in all sampling stations in the year 2016 [2], although even from among the ~5,000 isolates obtained we could not successfully grow in pure culture the major representatives of the genus *Winogradskyella* in the Helgoland Roads. As inferred from the phenotype of the *Candidatus* “*Winogradskyella atlantica*” MAGs, there was no objective reason for this failure, since this species would not be a particularly special heterotroph. Perhaps increased cultivation effort would have helped, or the use of multiple media and conditions. However, the question is whether the effort in cultivating and formally classifying an organism whose genome has been sequenced and binned on multiple occasions and for which we can infer a good relevant part of its phenotype, would have increased the scientific value that compensates for the time, effort and economic investment. We believe that the advance of a universal taxonomic framework cannot be hindered by the need for species in pure culture when their genomes can be accessed.

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Acknowledgements:

This study was funded by the Spanish Ministry of Sciences, Innovation and Universities projects PHRYEN_CLG2015_66686-C3-1-P, MARBIOM_RTC-2017-6405-1 and MICROMATES_PGC2018-096956-B-C41, and was also supported by European Regional Development Fund (FEDER) funds. RA was financed by the Max Planck Society. RRM acknowledges the financial support of a sabbatical stay at Georgia Tech supported by the grant PRX18/00048 of the Ministry of Sciences, Innovation and Universities.

References:

- 1- Acosta-González, A., Rosselló-Móra, R., Marqués, S. (2013) Characterization of the anaerobic microbial community in oil-polluted subtidal sediments: aromatic biodegradation potential after the prestige oil spill. *Environ. Microbiol.* 15, 77-92.

- 2- Alejandre-Colomo, C., Harder, J., Fuchs, B.M., Rosselló-Móra, R., Amann, R. I. (2020) High-throughput cultivation of heterotrophic bacteria during a spring phytoplankton bloom in the North Sea. *Syst. Appl. Microbiol.* 43, 126066.
- 3- Alonso, C., Warnecke, F., Amann, R., Pernthaler, J. (2007) High local and global diversity of Flavobacteria in marine plankton. *Environ. Microbiol.* 9, 1253–1266.
- 4- Begum, Z., Srinivas, T.N.R., Manasa, P., Sailaja, B., Sunil, B., Prasad, S., Shivaji, S. (2013) *Winogradskyella psychrotolerans* sp. nov., a marine bacterium of the family Flavobacteriaceae isolated from Arctic sediment. *Int. J. Syst. Evol. Microbiol.* 63, 1646–1652.
- 5- Bernardet, J.F., Nakagawa, Y., Holmes, B. (2002) Subcommittee on the taxonomy of *Flavobacterium* and *Cytophaga*-like bacteria of the International Committee on Systematics of Prokaryotes. Proposed minimal standards for describing new taxa of the family Flavobacteriaceae and emended description of the family. *Int. J. Syst. Evol. Microbiol.* 52, 1049–1070.
- 6- Bowman, J.P. (2000). Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. *Int. J. Syst. Evol. Microbiol.* 50, 1861–1868.
- 7- Chen, C.-L., Maki, J.S., Rittschof, D., Lay-Ming Teo, S. (2013) Early marine bacterial biofilm on a copper-based antifouling paint. *International Biodeterioration & Biodegradation* 83, 71-76.
- 8- Christensen, H., Bisgaard, M., Frederiksen, W., Mutters, R., Kuhnert, P., Olsen, J.E. (2001) Taxonomic note: is characterization of a single isolate sufficient for valid publication of a new genus or species? Proposal on reformulation of recommendation 30b of the *Bacteriological Code*. *Int. J. Syst. Evol. Microbiol.* 51, 2221–2225.
- 9- Claasens, N.J., Volpers, M., Martins dos Santos, V.A.P., van der Oost, J., de Vos, W.M. (2012) Potential of proton-pumping rhodopsins: engineering photosystems into microorganisms. *Trends Biotechnol.* 31, 633-642.
- 10- Cowan, S.T., Steel, K.J. (2003). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- 11- Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32(5), 1792–1797.
- 12- Einen, J., Øvreas, L. (2006) *Flaviramulus basaltis* gen. nov., sp. nov., a novel member of the family Flavobacteriaceae isolated from seafloor basalt. *Int. J. Syst. Evol. Microbiol.* 56, 2455–2461.

- 499
500
501
502
503
- 13- Franco, A., Busse, H.-J., Schubert, P., Wilke, T., Kämpfer, P., Glaeser, S. (2018) *Winogradskyella pocilloporae* sp. nov. isolated from healthy tissue of the coral *Pocillopora damicornis*. Int. J. Syst. Evol. Microbiol. 68, 1689–1696.
- 504
505
- 14- Fu, X.T., Kim, S.M. (2010) Agarase: Review of major sources, categories, purification method, enzyme characteristics and applications. Mar. Drugs. 8, 200-218.
- 506
507
508
509
510
- 15- Guannel, M.L., Horner-Devine, M.C., Rocap, G. (2011) Bacterial community composition differs with species and toxigenicity of the diatom *Pseudo-nitzschia*. Aquat. Microb. Ecol. 64, 117–133.
- 511
512
513
514
515
- 16- Ivanova, E.P., Christen, R., Gorshkova, N.M., Zhukova, N.V., Kurilenko, V.V., Crawford, R.J., Mikhailov, V.V. (2010) *Winogradskyella exilis* sp. nov., isolated from the starfish *Stellaster equestris*, and emended description of the genus *Winogradskyella*. Int. J. Syst. Evol. Microbiol. 60, 1577–1580.
- 516
517
518
- 17- Kämpfer, P., Kroppenstedt, R.M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. Can. J. Microbiol. 42, 989–1005.
- 519
520
521
- 18- Kang, H., Kim, H., Joung Y, Joh, K. (2017) *Winogradskyella maritima* sp. nov., isolated from seawater. Int. J. Syst. Evol. Microbiol. 67, 3840–3845.
- 522
523
524
- 19- Kang, C.H., Lee, S.Y., Yoon, J.H. (2013) *Winogradskyella litorisediminis* sp. nov., isolated from coastal sediment. Int. J. Syst. Evol. Microbiol. 63, 1793–1799.
- 525
526
527
528
- 20- Kim, S.-J., Choi, Y.-R., Park, S.-J., Kim, J.-G., Shin, K.-S., Roh, D.-H., Rhee, S.-K. (2013) *Winogradskyella pulchriflava* sp. nov., isolated from marine sediment. Int. J. Syst. Evol. Microbiol. 63, 3062–3068.
- 529
530
531
- 21- Kim, S.B., Nedashkovskaya, O.I. (2010) *Winogradskyella pacifica* sp. nov., a marine bacterium of the family *Flavobacteriaceae*. Int. J. Syst. Evol. Microbiol. 60, 1948–1951.
- 532
533
534
- 22- Kim, J.Y., Oh, D.C. (2012) *Winogradskyella jejuensis* sp. nov., a marine bacterium isolated from a brown alga *Carpopeltis affinis*. J. Microbiol. 50, 888–892.
- 535
536
537
538
- 23- Kim, J.-Y., Park, S.-H., Seo, G.-Y., Kim, Y.-J., Oh, D.-C. (2015) *Winogradskyella eckloniae* sp. nov., a marine bacterium isolated from the brown alga *Ecklonia cava*. Int. J. Syst. Evol. Microbiol. 65, 2791–2796.
- 539
540
541
- 24- Konstantinidis, K., Rosselló-Móra, R. (2015) Classifying the uncultivated microbial majority: a place for metagenomic data in the *Candidatus* proposal. Syst. Appl. Microbiol. 38, 223-230.

- 25- Konstantinidis, K., Rossello-Mora, R., Amann, R. (2017) Uncultivated microbes in need of their own taxonomy. *ISMEJ* 11, 2399-2406
- 26- Konstantinidis, K., Rosselló-Móra, R., Amann, R. (2020) Advantages outweigh concerns about using genome sequence as type material for prokaryotic taxonomy. *Environ. Microbiol.* 22, 819–822.
- 27- Konstantinidis, K.T., Tiedje, J.M. (2005) Towards a genome-based taxonomy for prokaryotes. *J. Bacteriol.* 187, 6258-6264.
- 28- Kruger, K., Chafee, M., Francis, T.B., Glavina del Rio, T., Becher, D., Schweder, T., Amann, R., Teeling, H. (2019) In marine *Bacteroidetes* the bulk of glycan degradation during algae blooms is mediated by few clades using a restricted set of genes. *ISME J.*, 1–17.
- 29- Kurilenko, V.V., Romanenko, L.A., Isaeva, M.P., Svetashev, V.I., Mikhailov, V.V. (2019) *Winogradskyella algae* sp. nov., a marine bacterium isolated from the brown alga. *Antonie van Leeuwenhoek.* 112, 731–739.
- 30- Lasica, A.M., Ksiazek, M., Madej, M., Potempa, J. (2017) The Type IX secretion system (T9SS): Highlights and recent insights into its structure and function. *Front. Cell. Infect. Microbiol.* 7, 215.
- 31- Lau, S.C., Tsoi, M.M., Li, X., Plakhotnikova, I., Dobretsov, S., Lau, K.W.K., Wu, M., Wong, P.-K., Pawlik, J.R., Qian, P.-Y. (2005) *Winogradskyella poriferorum* sp. nov., novel member of the family *Flavobacteriaceae* isolated from a sponge in the Bahamas. *Int. J. Syst. Evol. Microbiol.* 55, 1589–1592.
- 32- Lee, D.-H., Cho, S.J., Kim, S.M., Lee, S.B. (2013) *Winogradskyella damuponensis* sp. nov., isolated from seawater. *Int. J. Syst. Evol. Microbiol.* 63, 321–326.
- 33- Lee, J.H., Kang, J.W., Shin, S.B., Seong, C.N. (2017) *Winogradskyella flava* sp. nov., isolated from the brown alga, *Sargassum fulvellum*. *Int. J. Syst. Evol. Microbiol.* 67, 3540–3546.
- 34- Lee, S.Y., Park, S., Oh, T.K., Yoon, J.H. (2012) *Winogradskyella aquimaris* sp. nov., isolated from seawater. *Int. J. Syst. Evol. Microbiol.* 62, 1814–1818.
- 35- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadukumar, Buchner, A., Lai, T., Steppi, S., Jobb, G., Forster, W., Brettske, I., Gerber, S., Ginhart, A.W., Gross, O., Grumann, S., Hermann, S., Jost, R., König, A., Liss, T., Lussmann, T., May, M., Nonhoff, B., Reichel, B., Strehlow, R., Stamatakis, A., Stuckmann, N., Vildig, A., Lenke, M., Ludwig, T., Bode, A., Schleifer, K.H. (2004) ARB; a software environment for sequence data. *Nucleic Acids Res.* 32, 1363-1371.

- 36- Murray, A.E., Freudenstein, J., Gribaldo, S., Hatzenpichler, R., Hedlund, B.P., Hugenholtz, P., Kämpfer, P., Konstantinidis, K.T., Lane, C. E., Papke, R.T., Parks, D.H., Reysenbach, A.-L., Rosselló-Móra, R., Stott, M.B., Sutcliffe, I.C., Thrash, J.C., Venter, S.N., Whitman, W.B. et al., (2020) Roadmap for naming uncultivated Archaea and Bacteria. *Nature Microbiol.* IN PRESS
- 37- Nedashkovskaya, O.I., Kim, S.B., Han, S.K., Snauwaert, C., Vancanneyt, M., Swings, J., Kim, K.O., Lysenko, A.M., Rohde, M., Frolova, G.M., Mikhailov, V.V., Bae, K.S. (2005) *Winogradskyella thalassocola* gen. nov., sp. nov., *Winogradskyella epiphytica* sp. nov. and *Winogradskyella eximia* sp. nov., marine bacteria of the family *Flavobacteriaceae*. *Int. J. Syst. Evol. Microbiol.* 55, 49–55.
- 38- Nedashkovskaya, O.I., Kухлевский, A.D., Zhukova, N.V. (2012) *Winogradskyella ulvae* sp. nov., an epiphyte of a Pacific seaweed, and emended descriptions of the genus *Winogradskyella* and *Winogradskyella thalassocola*, *Winogradskyella echinorum*, *Winogradskyella exilis* and *Winogradskyella eximia*. *Int. J. Syst. Evol. Microbiol.* 62, 1450–1456.
- 39- Nedashkovskaya, O.I., Kухлевский, A.D., Zhukova, N.V., Kim, S.-J., Rhee, S.-K., Mikhailov, V.V. (2015) *Winogradskyella litoriviva* sp. nov., isolated from coastal seawater. *Int. J. Syst. Evol. Microbiol.* 65, 3652–3657.
- 40- Nedashkovskaya, O.I., Vancanneyt, M., Kim, S.B., Zhukova, N. V. (2009) *Winogradskyella echinorum* sp. nov., a marine bacterium of the family *Flavobacteriaceae* isolated from the sea urchin *Strongylocentrotus intermedius*. *Int. J. Syst. Evol. Microbiol.* 59, 1465–1468.
- 41- Park, S., Park, J.-M., Won, S.-M., Yoon, J.-H. (2015) *Winogradskyella crassostreae* sp. nov., isolated from an oyster (*Crassostrea gigas*) *Int. J. Syst. Evol. Microbiol.* 65, 2890–2895.
- 42- Park, S., Park, J.-M., Won, S.-M., Bae, K.S., Yoon, J.-H. (2014) *Winogradskyella wandonensis* sp. nov., isolated from a tidal flat. *Int. J. Syst. Evol. Microbiol.* 64, 1520–1525.
- 43- Park, S., Yoon, J.H. (2013) *Winogradskyella undariae* sp. nov., a member of the family *Flavobacteriaceae* isolated from a brown algae reservoir. *Antonie van Leeuwenhoek* 104, 619–626.
- 44- Parker, C.T., Tindall, B.J., Garrity, G.M. (2019) International Code of Nomenclature of Prokaryotes. *Int. J. Syst. Evol. Microbiol.* 69, S1 – S111
- 45- Parte, A. (2018) LPSN—List of Prokaryotic Names with Standing in Nomenclature (bacterio.net), 20 years on. *Int. J. Syst. Evol. Microbiol.* 68, 1825–1829.

- 46- Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., Glöckner, F.O. (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res.* 35, 7188-7196.
- 47- Reintjes, G., Fuchs, B.M., Scharfe, M., Wiltshire, K.H., Amann, R., Arnosti, C. (2020) Short-term changes in polysaccharide utilization mechanisms of marine bacterioplankton during a spring phytoplankton bloom. *Environ. Microbiol.* 22, 1884–1900.
- 48- Richter, M., Rosselló-Móra, R. (2009) Shifting the genomic gold standard for the prokaryotic species definition. *Proc. Nat. Acad. Sci. USA.* 106, 19126-19131.
- 49- Rodriguez-R., L.M., Konstantinidis, K.T. (2016) The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. *PeerJ. Preprints.* 4, e1900v1.
- 50- Rodriguez-Valera, F., Ventosa, A., Juez, G., Imhoff, J.F. (1985) Variation of environmental features and microbial populations with salt concentrations in a multi-ponds saltern. *Microbial Ecol.* 11, 107-115.
- 51- Romanenko, L.A., Kurilenko, V.V., Guzev, K.V., Svetashev, V.I., Mikhailov, V.V. (2019) *Winogradskyella profunda* sp. nov. isolated from the Chukchi Sea bottom sediments. *Arch. Microbiol.* 201, 45–50.
- 52- Romanenko, L.A., Tanaka, N., Frolova, G.M., Mikhailov, V.V. (2009) *Winogradskyella arenosi* sp. nov., a member of the family *Flavobacteriaceae* isolated from marine sediments from the Sea of Japan. *Int. J. Syst. Evol. Microbiol.* 59, 1443–1446.
- 53- Rosselló-Móra, R., Amann, R. (2015) Past and future species definitions for Bacteria and Archaea. *Syst. Appl. Microbiol.* 38, 209-216.
- 54- Rosselló-Móra, R., Konstantinidis, K.T., Sutcliffe, I., Whitman, W.B. (2020) Opinion: Response to concerns about the use of DNA sequences as types in the nomenclature of prokaryotes. *Syst. Appl. Microbiol.* 43, 126070.
- 55- Saitou, N., Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4, 406-425.
- 56- Schellenberg, J., Busse, H.-J., Hardt, M., Schubert, P., Wilke, T., Kämpfer, P., Glaeser, S.P. (2017) *Winogradskyella haliclona* sp. nov., isolated from a marine sponge of the genus *Haliclona*. *Int. J. Syst. Evol. Microbiol.* 67, 4902–4910.
- 57- Song, J., Lim, Y., Jang, H.-J., Young, Y., Kang, I., Hong, S.-J., Lee, C.-G., Cho, J.-C. (2019) Isolation and genome analysis of *Winogradskyella algicola* sp. nov., the dominant bacterial species associated with the green alga *Dunaliella tertiolecta*. *J. Microbiol.* 11, 982–990.

- 58- Song, J., Jeon, H.T., Lim, Y., Joung, Y., Cho, J.-C. (2018) *Winogradskyella aurantiaca* sp. nov., isolated from seawater. *Int. J. Syst. Evol. Microbiol.* 68, 3260–3265.
- 59- Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics.* 22, 2688-2690.
- 60- Sun, Y., Chen, B.-Y., Du, Z.-J. (2017) *Winogradskyella aurantia* sp. nov., isolated from a marine solar saltern. *Antonie van Leeuwenhoek.* 110, 1445–1452.
- 61- Tamames, J., Rosselló-Móra, R. (2012) On the fitness of microbial taxonomy. *Trends Microbiol.* 20, 514-516.
- 62- Valdenegro-Vega, V., Naeem, S., Carson, J., Bowman, J.P., Tejedor del Real, J.L., Nowak, B. (2013) Culturable microbiota of ranched southern blue-fin tuna (*Thunnus maccoyii* Castelnau). *J. Appl. Microbiol.* 115, 923–932.
- 63- Vandecandelaere, I., Nercessian, O., Faimali, M., Segaeert, E., Mollica, A., De Vos, P., Vandamme, P. (2010) Bacterial diversity of the cultivable fraction of a marine electroactive biofilm. *Bioelectrochemistry.* 78, 62–66.
- 64- Viver, T., Cifuentes, A., Díaz, S., Rodríguez-Valdecantos, G., González, B., Antón, J., Rosselló-Móra, R. (2015) Diversity of extremely halophilic cultivable prokaryotes in Mediterranean, Atlantic and Pacific solar salterns: evidence that unexplored sites constitute sources of cultivable novelty. *Syst. Appl. Microbiol.* 38, 266-275.
- 65- Viver, T., Orellana, L.H., Díaz, S., Urdiain, M., Ramos-Barbero, M.D., Gonzalez-Pastor, J.E., Oren, A., Hatt, J. K., Amann, R., Anton, J., Konstantinidis, K.T., Rosselló-Móra, R. (2019) Predominance of deterministic microbial community dynamics in salterns exposed to different light intensities. *Environ. Microbiol.* 21, 4300–4315.
- 66- Viver, T., Orellana, L., Gonzalez-Torres, P., Diaz, S., Urdiain, M., Fariás, M.E., Benes, V., Kämpfer, P., Shahinpei, A., Amoozegar, M.A., Amann, R., Anton, J., Konstantinidis, K.T., Rosselló-Móra R (2018) Genomic comparison between members of the *Salinibacteraceae* family, and description of a new species of *Salinibacter* (*Salinibacter altiplanensis* sp. nov.) isolated from high altitude hypersaline environments of the Argentinian Altiplano. *Syst. Appl. Microbiol.* 41, 198-212.
- 67- Wang, C., Han, J.-R., Liu, C.-L., Du, Z.-J. (2018) *Winogradskyella tangerina* sp. nov., a member of the *Flavobacteriaceae* isolated from coastal sediment. *Int. J. Syst. Evol. Microbiol.* 68, 2832–2837.
- 68- Whitman, W.B. (2015). Genome sequences as the type material for taxonomic descriptions of prokaryotes. *Syst. Appl. Microbiol.* 38, 217-222.

- 69- Whitman, W.B. (2016). Modest proposals to expand the type material for naming of prokaryotes. *Int. J. Syst. Evol. Microbiol.* 66, 2108-2112.
- 70- Whitman, W.B., Sutcliffe, I., Rossello-Mora, R. (2019) Proposal for changes in the International Code of Nomenclature of Prokaryotes: granting priority to *Candidatus* names. *Int. J. Syst. Evol. Microbiol.* 69, 2174–2175.
- 71- Yoon, B.-J., Byun, H.-D., Kim, J.-Y., Lee, D.-H., Kahng, H.-Y., Oh, D.-C. (2011) *Winogradskyella lutea* sp. nov., isolated from seawater, and emended description of the genus *Winogradskyella*. *Int. J. Syst. Evol. Microbiol.* 61, 1539–1543.
- 72- Yoon, J.-H., Lee S.-Y. (2012) *Winogradskyella multivorans* sp. nov., a polysaccharide degrading bacterium isolated from seawater of an oyster farm. *Antonie van Leeuwenhoek*. 102, 231–238.
- 73- Zhang, D.-C., Liu, Y.-X., Huang, H.-J., Weber, K., Margesin, R. (2016) *Winogradskyella sediminis* sp. nov., isolated from marine sediment. *Int. J. Syst. Evol. Microbiol.* 66, 3157–3163.

Figure and table legends

Figure 1: Phylogenetic reconstruction based on the 16S rRNA gene sequences of all *Winogradskyella* species. The tree was reconstructed using maximum likelihood analysis and is the result of the consensus of different approaches using distinct filters and datasets. The multifurcations indicate a branching order that could not be resolved. Bar indicates 10% sequence divergence.

Con formato: Inglés (Estados Unidos)

Figure 2: Phylogenetic reconstruction based on the concatenated core genome sequence of all *Winogradskyella* species and MAGs for which the genome was available. The tree was reconstructed using maximum likelihood analysis. Bar indicates 10% sequence divergence.

Con formato: Inglés (Estados Unidos)

Table 1: Diagnostic phenotypes of all *Winogradskyella* species.

Table 2: Pairwise whole genome comparisons at the nucleotide level (ANI, upper triangle) and protein translated genes (AAI, lower triangle). The blue shaded color represents the new species classified in this study, and the species already classified are shown in orange.

Table 3: Fatty acid profiles of the new isolates in comparison with the available *Winogradskyella* type strain profiles. Profiles labelled with (+) were determined in this study.

Table 4: Protologues of the new species descriptions.

Con formato: Inglés (Estados Unidos)