

# Phylogenetic heterogeneity within the genus *Herpetosiphon*: transfer of the marine species *Herpetosiphon cohaerens*, *Herpetosiphon nigricans* and *Herpetosiphon persicus* to the genus *Lewinella* gen. nov. in the *Flexibacter–Bacteroides–Cytophaga* phylum

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**Analysis of the 16S rDNA sequences of species currently assigned to the genus *Herpetosiphon* revealed intragenetic phylogenetic heterogeneity. The thermotolerant freshwater species *Herpetosiphon geysericola* is most closely related to the type species *Herpetosiphon aurantiacus* in the *Chloroflexus* subdivision of the green non-sulfur bacteria. The marine species *Herpetosiphon cohaerens*, *Herpetosiphon nigricans* and *Herpetosiphon persicus*, on the other hand, were found to form a cluster with the sheathed bacterium *Haliscomenobacter hydrossis* in the *Saprospira* group of the *Flexibacter–Bacteroides–Cytophaga* (FBC) phylum. A proposal is made to transfer these marine species to the genus *Lewinella* gen. nov. as *Lewinella cohaerens* comb. nov., *Lewinella nigricans* comb. nov. and *Lewinella persica* comb. nov. The marine sheathed gliding bacterium *Flexithrix dorotheae* was also found to be a member of the FBC phylum but on a separate phylogenetic line to the marine herpetosiphons now assigned to the genus *Lewinella*.**

**Keywords:** *Herpetosiphon*, *Lewinella* gen. nov., *Flexibacter–Bacteroides–Cytophaga* phylum

## INTRODUCTION

The genus *Herpetosiphon* currently contains five species (25, 41) of gliding bacteria characterized by the ability to form sheathed filaments (9, 20). The genus and the type species *Herpetosiphon aurantiacus* were first described in 1968 by Holt & Lewin (10). The genus was assigned to the order *Flexibacterales* as defined by Soriano & Lewin (43), and it was considered that *H. aurantiacus* was an apochlorotic counterpart of the cyanobacterium *Lyngbya*, supported at the time by similar base composition of their DNA (24). In 1970, three new marine species of *Herpetosiphon*, *Herpeto-*

*siphon cohaerens*, *Herpetosiphon nigricans* and *Herpetosiphon persicus*, were described and a fourth species incorrectly classified as the cyanobacterium *Phormidium geysericola* was transferred to the genus as *Herpetosiphon geysericola* comb. nov (20). Lewin (19) also described another flexuous gliding ensheathed marine species, *Flexithrix dorotheae*, which is distinguished by its propensity for false branching and a lower DNA base composition. The DNA base composition of this species was 37.2 mol% G + C, which is well below the 45–53 mol% G + C range for the species of *Herpetosiphon* (24). *Flexithrix dorotheae* and the marine species of *Herpetosiphon* apparently have not been studied in detail since and very little new information is known about their characteristics. *H. aurantiacus* is known to belong to the *Chloroflexus* subdivision of the green non-sulfur bacteria (7, 30, 50) but the intragenetic relationships of the species of *Herpetosiphon* have been based on phenotypic characteristics (9, 10, 20, 36) and DNA base composition data (24). In this paper we report the determination

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**Abbreviation:** FBC, *Flexibacter–Bacteroides–Cytophaga*.

The GenBank accession numbers for the rDNA sequences of *Herpetosiphon geysericola* ATCC 23076<sup>T</sup>, *Lewinella cohaerens* ATCC 23123<sup>T</sup>, *Lewinella nigricans* ATCC 23147<sup>T</sup>, *Lewinella persica* ATCC 23167<sup>T</sup> and *Flexithrix dorotheae* ATCC 23163<sup>T</sup> reported in this paper are AF039292–AF039296, respectively.

and analysis of the 16S rDNA sequences for the type strains of the species currently assigned to the genus *Herpetosiphon*, which revealed that the marine species are phylogenetically unrelated to the freshwater species, including the type species of the genus, *H. aurantiacus*. Consequently, we propose that these marine species be transferred to the genus *Lewinella* gen. nov. named in honour of Professor Ralph Lewin who first described these bacteria.

## METHODS

**Bacterial cultures.** Cultures of *Herpetosiphon cohaerens* ATCC 23123<sup>T</sup>, *Herpetosiphon geysericola* ATCC 23076<sup>T</sup>, *Herpetosiphon nigricans* ATCC 23147<sup>T</sup>, *Herpetosiphon persicus* ATCC 23167<sup>T</sup> and *Flexithrix dorotheae* ATCC 23163<sup>T</sup> were obtained from the American Type Culture Collection, Rockville, MD, USA.

**16S rDNA sequencing methods.** Direct PCR amplification of 16S rDNA was performed as previously described (46). The PCR products were purified using Promega Magic PCR Prep DNA Purification according to the manufacturer's instructions. The PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems) was used with the primers 27f, 926f, 1114f, 357r, 519r, 907r, 1100r, 1392r and 1525r (15) and 787r and 803f (44) to directly sequence the PCR products, following the protocol provided by the manufacturer. The reaction mixtures were sequenced automatically on an Applied Biosystems 373A DNA Sequencer.

**Data analysis.** The 16S rDNA sequences were aligned manually using the ae2 editor with representative bacterial 16S rRNA gene sequences from the RNA Database Project (22) and GenBank. Positions where length and sequence variations made alignment uncertain were omitted from the analysis. Pairwise evolutionary distances were computed using the method of Jukes & Cantor (13) in the DNADIST program in the PHYLIP 3.5c software package (5). Phylogenetic dendrograms were constructed using the neighbour-joining method of Saitou & Nei (40) and by the parsimony method using DNAPARS in PHYLIP. Bootstrap analyses using SEQBOOT PHYLIP 3.5c were performed to determine the statistical significance of branching patterns and consensus trees (not shown) were produced using CONSENSE (PHYLIP 3.5c).

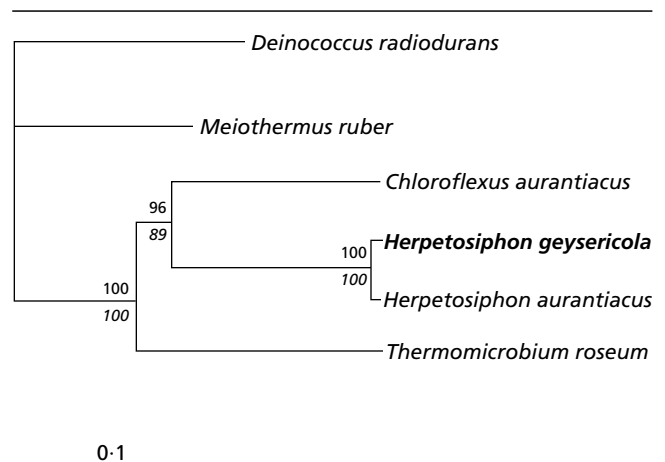
Reference sequence accession numbers and strain numbers (where available) used in the phylogenetic analyses were as follows: *Bacteroides fragilis*, M61006; *Chlorobium vibriiforme* DSM 260<sup>T</sup>, M62791; *Chloroflexus aurantiacus* ATCC 29366<sup>T</sup>, M34116; *Cyclobacterium marinus* ATCC 43824, M62788; *Cytophaga diffluens* ATCC 23140, M58765; *Cytophaga hutchinsonii* ATCC 33406<sup>T</sup>, M58768; *Deinococcus radiodurans* ATCC 35073, M21413; *Empedobacter brevis* ATCC 14234, M59052; *Flavobacterium aquatile* ATCC 11947<sup>T</sup>, M62797; *Flavobacterium ferrugineum* ATCC 13524<sup>T</sup>, M62798; *Flectobacillus major* ATCC 29496<sup>T</sup>, M62787; *Flexibacter aggregans* ATCC 23162<sup>T</sup>, M64628; *Flexibacter canadensis* ATCC 29591<sup>T</sup>, M62793; *Flexibacter litoralis* ATCC 23117<sup>T</sup>, M58784; *Flexibacter roseolus* ATCC 23088<sup>T</sup>, M58787; *Flexibacter ruber* ATCC 23103<sup>T</sup>, M58788; *Flexibacter sancti* ATCC 23092<sup>T</sup>, M62795; *Haliscomenobacter hydrossis* ATCC 27775<sup>T</sup>, M58790; *Herpetosiphon aurantiacus* ATCC 23779<sup>T</sup>, M34117; *Meiothermus ruber* ATCC 35948<sup>T</sup>, Z15059; '*Microscilla aggregans* subsp. *catalatica*' ATCC 23190, M58791; '*Microscilla arenaria*'

ATCC 23161, M60455; '*Microscilla furvescens*', ATCC 23129, M58792; *Rhodothermus marinus*, X77140; *Runella slithyiformis* ATCC 29530<sup>T</sup>, M62786; *Saprospira grandis* ATCC 23119<sup>T</sup>, M58795; *Spirosoma linguale* ATCC 23276, M62789; *Thermomicrobium roseum* ATCC 27502<sup>T</sup>, M34115; and *Thermonema lapsum*, L11703.

## RESULTS AND DISCUSSION

Comparison of the 16S rDNA sequences of the species of *Herpetosiphon* has revealed phylogenetic heterogeneity within the genus unexpected from previous limited knowledge of their phenotypic and genotypic characteristics. Almost complete 16S rDNA sequences (> 1417 nucleotides) were obtained for the type strains of the species *H. cohaerens*, *H. geysericola*, *H. nigricans* and *H. persicus*. The 16S rDNA sequences were compared with the known sequence available for the type strain of *H. aurantiacus* and other reference sequences required to phylogenetically position the species of *Herpetosiphon*.

Knowledge of the phylogenetic position of the genus *Herpetosiphon* has been based on data obtained for the type species *H. aurantiacus*. Evidence obtained from 16S rRNA oligonucleotide catalogues (7, 50) and 16S rRNA sequences (30) has shown that this species belongs to the green non-sulfur bacteria together with *Chloroflexus* and *Thermomicrobium*. This relationship between *Herpetosiphon* and *Chloroflexus* is supported by their unusual cell wall composition (11, 12). The analysis of the 16S rDNA sequences for the other *Herpetosiphon* species obtained in the present study showed that the thermotolerant freshwater species *H. geysericola* was phylogenetically related to *H. aurantiacus* in the green non-sulfur phylum, but that the



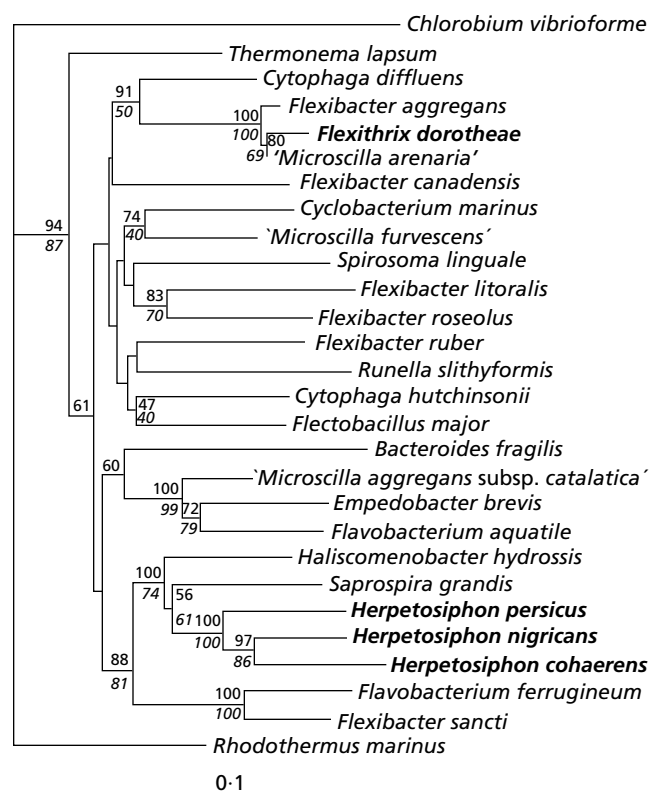
**Fig. 1.** Phylogenetic tree constructed by the neighbour-joining method showing the relationships of species of the genus *Herpetosiphon* in the green non-sulfur phylum. The sequence of *Deinococcus radiodurans* was used as the outgroup. Bootstrap values of 100 analyses are shown at the top of the branching points for the neighbour-joining analysis and below (in italics) for the parsimony analysis. The scale bar represents 10 nucleotide substitutions per 100 nucleotides of 16S rDNA sequence.

three marine species *H. cohaerens*, *H. nigricans* and *H. persicus* belong to the *Flexibacter*–*Bacteroides*–*Cytophaga* (FBC) phylum.

The dendrogram of relationships (Fig. 1) inferred from a neighbour-joining analysis of the corrected dissimilarity values over a length of 1058 nucleotides indicated that *H. geysericola* is most closely related to *H. aurantiacus* amongst the members of the green non-sulfur bacteria. The sequences of *H. aurantiacus* and *H. geysericola* have a similarity of 96.4%, which is indicative of separate species status (45, 48) within the genus. The analysis of the 16S rDNA sequence data by the parsimony method produced a phylogenetic tree (not shown) with the same topography as the neighbour-joining tree. Bootstrap values determined from 100 resamplings indicate strong statistical support for the phylogenetic relationships.

For the marine species *H. cohaerens*, *H. nigricans* and *H. persicus*, the dendrogram of relationships (Fig. 2) inferred from a neighbour-joining analysis of the corrected dissimilarity values over a length of 1105 nucleotides showed that these species clustered together in a deep phylogenetic line within the *Saprospira* group of the FBC phylum. The *Saprospira* group also includes the non-motile sheathed organism *Haliscomenobacter hydrossis* (3, 6, 47). *Haliscomenobacter hydrossis* is not a marine organism but rather is isolated from sewage treatment plants where it often causes problems with bulking (3, 47). The 16S rDNA sequences over a length of 1418 nucleotides have similarity values in the range 86–88%, which is clearly indicative of separate species status in spite of the few known phenotypic characteristics which differentiate these species (9, 20, 36). Bootstrap values provide strong support that the marine *Herpetosiphon* species form a phylogenetically coherent cluster within the well-supported *Saprospira* group (Fig. 2).

The present research findings confirm the taxonomic and phylogenetic relationships of the marine species of the genus *Herpetosiphon*, but not the type species of the genus, with the flexibacteria, now known to be members of the FBC phylum. These findings emphasize that the characteristics of gliding motility and ensheathed filament formation are not phylogenetically discriminative features (39). The phylogenetic position of the genus *Herpetosiphon* has been a matter of speculation since its description, and at various times these organisms have been considered to be apochlorotic counterparts of the cyanobacteria (10, 20, 37), *Flexibacteriaceae* (18), *Cytophagaceae* (17, 18), and currently as a separate genus in the green non-sulfur bacteria (36). Chemotaxonomic determination of sulfonolipids (8) and the absence of flexirubin-like pigments (14, 38) refuted a close relationship with the cytophagas. However, these studies have been restricted to the type species *H. aurantiacus* or its synonymous relative '*Herpetosiphon giganteus*'. The marine species remain poorly studied and their in-



**Fig. 2.** Phylogenetic tree constructed by the neighbour-joining method showing the relationships of species of the genus *Herpetosiphon* and *Flexithrix dorotheae* in the FBC phylum. The sequence of *Chlorobium vibrioforme* was used as the outgroup. Bootstrap values of 100 analyses are shown at the top of the branching points for the neighbour-joining analysis and below (in italics) for the parsimony analysis. The scale bar represents 10 nucleotide substitutions per 100 nucleotides of 16S rDNA sequence.

clusion in the chemotaxonomic investigations may have provided an earlier insight into their relationship with the FBC bacteria. Likewise, determination of the phylogenetic position of the genus by 16S rRNA oligonucleotide catalogue analysis (7, 39, 50) and 16S rRNA sequences (30) was also confined to data from *H. aurantiacus*. These studies showed that the type species, and therefore the genus *Herpetosiphon*, is most closely related to *Chloroflexus* and *Thermomicrobium*. Like *H. aurantiacus*, the phototrophic gliding filamentous bacterium *Chloroflexus* may be thinly sheathed (1, 32) and has an unusual cell wall structure and composition. Both organisms lack LPS and contain a polysaccharide–peptidoglycan complex in which *meso*-diaminopimelic acid is replaced by L-ornithine (11, 12, 36). Interestingly, Pierson & Castenholz in their description of *Chloroflexus aurantiacus* (32) observed that there was a striking morphological resemblance to some filamentous gliding flexibacteria such as *Herpetosiphon* which have similar DNA G + C composition (20, 24), but cautioned that this observation may be coincidental. It is probable that gliding motility and ensheathed morphology were early evolutionary traits and bacteria with these shared

characteristics have now been observed in three evolutionary lineages, including the green non-sulfur bacteria, the FBC bacteria and the cyanobacteria.

The existence of a sheath in *Herpetosiphon* has been a matter of disagreement at least in part due to differences in interpretation of this morphological feature, possibly due to variation in growth conditions and methods of examination. Holt & Lewin (10) included the presence of a sheath in the description of *Herpetosiphon* and confirmed this in the subsequent description of additional species (20). However, Reichenbach & Golecki (37) disputed the presence of a classical sheath, but acknowledge the existence of an outer membranous structure they referred to as a 'sleeve'. Plausible arguments were mounted against the existence of a sheath (37), but some electron micrographs of thin sections of *H. aurantiacus* (9, 42) support the existence of a sheath surrounding the cells.

Following our finding that the marine species of *Herpetosiphon* belong to the FBC phylum, which includes many marine species of gliding bacteria, we considered it necessary to determine the relationship of these species with the other marine gliding sheathed bacterium, *Flexithrix dorotheae*, which was also isolated and described by Lewin (19) and for which a 16S rRNA gene sequence was not available. The almost complete sequence was determined and phylogenetic analysis (Fig. 2) showed that this bacterium is in fact a member of the FBC phylum but does not group with the marine herpetosiphons. Rather, *Flexithrix dorotheae* clusters on a separate phylogenetic line with the marine species *Flexibacter aggregans* and '*Microscilla arenaria*'. The 16S rDNA sequence of *Flexithrix dorotheae* has a similarity of 97.8% with that of its closest relative '*Microscilla arenaria*' and 96% with *Flexibacter aggregans* over a sequence length of 1460 nucleotides. The sequences of *Flexibacter aggregans* and '*Microscilla arenaria*' have a similarity of 98.1%. Recently, Nakagawa *et al.* (28) showed that *Flexibacter aggregans* is only distantly related to the type species of the genus *Flexibacter*, *Flexibacter flexilis*, and its taxonomic position is therefore uncertain. Because of the high 16S rRNA gene sequence similarities between *Flexithrix dorotheae*, *Flexibacter aggregans*, and '*Microscilla arenaria*', DNA-DNA homology values will be required to determine their separate species status. It is possible that all three species belong to the one genus in which sheath formation is not a common characteristic. Lewin (19) proposed the genus *Flexithrix* to include the single species *Flexithrix dorotheae* characterized by flexuous cells capable of gliding but which formed falsely branched sheathed filaments. Whether the cultures consisted predominantly of ensheathed filaments or individual gliding cells was dependent on the cultural conditions (19, 34). This species was considered to be distinct from the marine ensheathed species Lewin assigned to the genus *Herpetosiphon* on the basis of sheathed morphology and substantially lower DNA base composition.

The close phylogenetic association of *Flexithrix dorotheae* with *Flexibacter aggregans* and '*Microscilla arenaria*' highlights the persistent uncertainty which has beset the taxonomy of the members of the flexibacteria over a long period of time. Lewin (18) considered the flexibacteria to belong to four groups consisting of the *Cytophaga-Microscilla-Flexibacter* group, *Saprospira*, *Flexithrix* and *Herpetosiphon*. Previous phylogenetic research (6, 28, 29, 49, 50, 51) has shown that *Cytophaga*, *Microscilla*, *Flexibacter* and *Saprospira* belong to the FBC phylum, and the present research has confirmed this relationship for *Flexithrix* and the marine species of *Herpetosiphon*. Although *Herpetosiphon* in recent times has been excluded from taxonomic treatments of the *Cytophagaceae* in the *Cytophagales* (33, 35), presumably because of the phylogenetic relationship of the type species with *Chloroflexus* in the green non-sulfur bacteria, *Flexithrix* has continued to be included (33, 34, 35) and the present findings justify this taxonomic judgement. Lewin's taxonomic reorganization of the flexibacteria was largely consistent with the findings of Fager's (4) recurrent group analysis of phenotypic characters, except that Lewin considered sheath formation to be such an important recognizable characteristic that it led him to separate strain QQ-3 (ATCC 23163<sup>T</sup>) from group 3 of Fager's assemblage and describe it as *Flexithrix dorotheae*. Interestingly, in light of the present phylogenetic study, the remaining strains in group 3 were named '*Microscilla aggregans*' and included *Flexibacter (Microscilla) aggregans* strain NN-13 (= ATCC 23162<sup>T</sup>) which we have shown is a close phylogenetic relative of *Flexithrix dorotheae* on the basis of 96% 16S rRNA gene sequence similarity. The type strain of *Flexithrix dorotheae* is reported as not frequently producing a sheath and may then easily be confused with the flexibacters and microscillas, particularly in liquid culture (34). In its unsheathed form *Flexithrix dorotheae* closely resembles *Microscilla (Flexibacter) aggregans* (34), a relationship which is strongly supported by the present phylogenetic results. In our hands, we were also unable to observe a sheath in this strain and agree that its morphology is very similar to the unsheathed flexibacters. On the basis of a numerical analysis of a limited range of phenotypic traits determined by Lewin & Lounsbury (21), Colwell (2) found insufficient support for the elevation of *Flexithrix dorotheae*, *Microscilla* species, and the marine *Herpetosiphon* species to generic rank and recommended that further study was required. The present study has provided phylogenetic evidence which supports the generic rank of *Flexithrix*, and also of the marine herpetosiphons, but has not considered the genus *Microscilla*, which has been addressed by others. The polyphyletic nature of the genus *Herpetosiphon* is not surprising given the frequent revelations on the phylogeny of the genera of the flexibacteria. Previous research has revealed the polyphyletic nature of the genera *Flexibacter*, *Microscilla* and *Cytophaga*, as well as showing their relationship with non-motile genera such *Flavobacterium*, *Bacteroides* and *Halisco-*

**Table 1.** Differential characteristics of the genus *Lewinella*, other genera of gliding bacteria that form ensheathed filaments and *Haliscomenobacter*

Data compiled from references 6, 7, 9, 16, 19, 20, 23, 24, 26, 27, 30, 32, 34, 36, 47 and this study. Abbreviations: +, positive; –, negative; +/- variable, FW, freshwater; M, marine; S, sewage; FBC, *Flexibacter*–*Bacteroides*–*Cytophaga* bacteria; GNS, green non-sulfur bacteria.

Characteristic	<i>Lewinella</i>	<i>Flexithrix</i>	<i>Haliscomenobacter</i>	<i>Herpetosiphon</i>	<i>Chloroflexus</i>	<i>Thiothrix</i>	<i>Thioploca</i>
Gliding motility	+	+	–	+	+	+	+
Sheath	Unbranched	Falsely branched	Branched	Unbranched	Unbranched	Branched	Unbranched
Holdfast	–	–	–	–	–	+	–
Chemo-organotroph	+	+	+	+	+/-	+/-	–
Phototroph	–	–	–	–	+	–	–
Mixotroph	–	–	–	–	–	+/-	+(?)
Carotenoid pigments	+	+	+	+	+	–	–
Bacteriochlorophyll	–	–	–	–	+	–	–
S deposits from H <sub>2</sub> S	–	–	–	–	–	+	+
Habitat	M	M	FW, S	FW, S	FW	FW, S, M	FW, M
Phylogenetic affiliation	FBC	FBC	FBC	GNS	GNS		
G + C content (mol %)	45–53	37	49	48	53–55	52	

*menobacter* in the FBC phylum (6, 28, 29, 31, 49, 50, 51).

It is clear that the marine species currently assigned to the genus *Herpetosiphon* can not be considered to be members of this genus as they are only distantly related to the type species. Consequently, we propose that a new genus be described to accommodate them, for which we propose the name *Lewinella* gen. nov. No further phenotypic characterization of these species has been undertaken and the description of the species is based on data from previous publications (9, 20, 24, 36). Characteristics useful for the differentiation of the genus *Lewinella* from other gliding filamentous sheathed bacteria, and *Haliscomenobacter* are given in Table 1.

#### Description of *Lewinella* gen. nov.

*Lewinella* (Le.win.el'la. M.L. dim. ending *-ella*; M.L. fem. dim. n. *Lewinella* named after Professor Ralph Lewin, who first isolated these organisms).

Unbranched, flexible rods or filaments 0.5–1.5 × 5–150 µm or more (to several millimetres) consisting of individual cells 2–3 µm long. Often ensheathed, although sheaths may be difficult to visualize. Filament breakage or cell lysis results in empty ends or spaces (necridia). Resting stages not known. Motile by gliding. Gram-negative. Produce orange or yellow carotenoid pigments. Chemo-organotrophs. Strict aerobes. Metabolism is respiratory, with molecular oxygen used as the terminal electron acceptor. Gelatin is hydrolysed, but not cellulose, chitin or starch. Require seawater for growth. Isolated from marine environments. Belongs to the FBC phylum. The DNA G + C content is 44.9–53.1 mol% (Bd) (24). The type species of the genus is *Lewinella cohaerens*. The species assigned to the genus *Lewinella* have not been studied extensively since their description by Lewin (20). The descriptions below are based on data and taxonomic

considerations from Lewin (20), Mandel & Lewin (24), Holt (9), Reichenbach (36) and the present study.

#### Description of *Lewinella cohaerens* (Lewin 1970) comb. nov.

*Lewinella cohaerens* (co.hae'rens. L. part. adj. *cohaerens* cohering, uniting together).

Unbranched, flexible, sheathed rods or filaments 0.7 µm (1.0 µm including sheath) × 60–150 µm or longer. Cell masses have an orange pigment believed to be the carotenoid saxopyxanthin. Glucose and sucrose promote growth, but acetate, galactose, glycerol and lactate do not. Tryptone and glutamate but not nitrate serve as nitrogen sources. No acid is produced in litmus milk, but the milk is coagulated and the litmus is reduced. Tyrosine or dihydroxyphenylalanine are not degraded. No growth factors are required. One-half to double-strength seawater is required for growth. The DNA G + C content of the type strain is 44.9 mol% (Bd). The type strain is strain II-2 (= ATCC 23123<sup>T</sup>) isolated from beach sand, Biarritz, France.

#### Description of *Lewinella persica* comb. nov.

*Lewinella persica* [per'si.ca. L. adj. *persica* Persian (of fruit = peach), i.e. peach-coloured].

Unbranched, flexible, sheathed rods or filaments 0.7 µm (1.0 µm including sheath) × 30–150 µm or longer. Cell masses have an orange pigment believed to be the carotenoid saxopyxanthin. Glucose is a suitable carbon source. Sucrose and galactose promote growth, but acetate, glycerol and lactate do not. Tryptone, glutamate or nitrate serve as sole nitrogen sources. No acid is produced in litmus milk, but the milk is coagulated and the litmus is reduced. Tyrosine or dihydroxyphenylalanine are not degraded. No growth factors are required. One-half to double-strength seawater is required for growth. The DNA G + C

content of the type strain is 52.6 mol % (Bd). The type strain is strain T-3 (= ATCC 23167<sup>T</sup>) isolated from brown mud, Galway, Ireland.

#### Description of *Lewinella nigricans* comb. nov.

*Lewinella nigricans* (ni'gri.cans. L. part. adj. *nigricans* blackening).

Unbranched, flexible, sheathed rods or filaments 0.5 µm (1.0 µm including sheath) × 5–50 µm. Cell masses have a yellow pigment believed to be the carotenoid zeaxanthin. Glucose is a suitable carbon source. Sucrose and galactose promote growth, but acetate, glycerol and lactate do not. Casamino acids, tryptone, glutamate or nitrate serve as sole nitrogen sources. No acid is produced in litmus milk, but the milk is coagulated and digested, and the litmus is reduced. Tyrosine is degraded with the formation of a pigmented product. Dihydroxyphenylalanine is not degraded. No growth factors are required. One-half to double-strength seawater is required for growth. The DNA G + C content of the type strain is 53.1 mol % (Bd). The type strain is strain SS-2 (= ATCC 23147<sup>T</sup>) isolated from beach sand near Lagos, Nigeria.

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