Gordonibacter pamelaeae gen. nov., sp. nov., a new member of the *Coriobacteriaceae* isolated from a patient with Crohn's disease, and reclassification of *Eggerthella hongkongensis* Lau *et al.* 2006 as *Paraeggerthella hongkongensis* gen. nov., comb. nov.

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A strictly anaerobic, Gram-positive, short-rod/coccobacillus-shaped bacterial strain, designated 7-10-1-b^T, was isolated from the colon of a patient suffering from acute Crohn's disease. The isolate formed small, pale-white, semi-translucent colonies on solid cultivation media. The strain was catalase-positive and metabolized only a small number of carbon sources. Whole-cell fatty acids consisted predominantly of saturated fatty acids (89 %), of which 15 : 0 anteiso was the major component. The polar lipids phosphatidylglycerol and diphosphatidylglycerol as well as four glycolipids were identified. 16S rRNA gene sequence analysis revealed that the isolate represents a distinct lineage within the family *Coriobacteriaceae* and has 94.6 % identity to the type strain of [*Eggerthella*] *hongkongensis*, the phylogenetically closest bacterial species. On the basis of the analyses performed, the new genus and species *Gordonibacter pamelaeae* gen. nov., sp. nov. is described, with strain 7-10-1-b^T (=DSM 19378^T =CCUG 55131^T) as the type and only strain of *Gordonibacter pamelaeae*. Also, based on the chemotaxonomic data obtained for all type strains of the neighbouring genus *Eggerthella*, we propose that *Eggerthella hongkongensis* Lau *et al.* 2006 be transferred to a new genus as *Paraeggerthella hongkongensis* gen. nov., comb. nov.; the type strain of *Paraeggerthella hongkongensis* is HKU10^T (=DSM 16106^T =CCUG 49250^T).

Abbreviation: DMA, dimethylacetal.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 7-10-1-b^T is AM886059.

A dendrogram derived from biochemical profiles, a maximum-likelihood tree based on 16S rRNA gene sequences, TLC of polar lipids of strain 7-10-1-b^T and comparisons of the fatty acid and menaquinone profiles of strain 7-10-1-b^T and related type strains are available as supplementary material with the online version of this paper.

During a study on the intestinal flora of patients with inflammatory bowel disease (IBD), one isolate from the colon of a patient with acute Crohn's disease showed a unique BOX-PCR fingerprint and subsequently a unique 16S rRNA gene sequence pattern, with 94.6–95.2 % similarity to members of the genus *Eggerthella*. The genus *Eggerthella* belongs to the family *Coriobacteriaceae*, placed within the class *Actinobacteria* (Wade *et al.*, 1999).

Members of this genus are anaerobic, non-sporulating, non-motile, Gram-positive bacilli that grow singly, as pairs or in short chains. They are found in the human colon and faeces and have been implicated as a cause of ulcerative colitis, liver and anal abscesses and systemic bacteraemia (Chan & Mercer, 2008; Landais *et al.*, 2007; Lau *et al.*, 2004a, b). The type species of the genus, *Eggerthella lenta*, was originally described as '*Bacteroides lentus*' (Eggerth, 1935), but was included on the Approved Lists of Bacterial Names in the genus *Eubacterium*, as *Eubacterium lentum*.

Growth characteristics

Strain 7-10-1-b^T was isolated from the sigmoid region of the colon of a patient with active Crohn's disease (male, age 33, medication Azathioprine + Mutaflor + cortisone) and was obtained by colonoscopy on 30 November 2006 in the Clinic for General Internal Medicine (University-Hospital Schleswig-Holstein, Kiel, Germany). Samples were placed in standard anaerobic transport medium (Port-a-cul tube; BBL) on ice and processed for isolation of bacteria within 24 h. One biopsy specimen was transferred to a microcentrifuge tube with 100 µl sterile PBS (50 mM potassium phosphate, 150 mM NaCl, pH 7.2) and homogenized by repeated grinding with a Teflon mortar. The homogenate was diluted further in PBS and aliquots corresponding to 10 % (1 : 10) and 1 % (1 : 100) of the original biopsy material were plated on a variety of different media. The isolation procedure and incubation of plates was carried out under anaerobic conditions (MACS-VA500 workstation; Meintrup DWS) with an atmosphere consisting of $N_2/H_2/CO_2$ (80 : 10 : 10) at 37 °C. Plates were monitored for growth and single colonies were subcultivated, checked for purity and screened by BOX-PCR as described previously (Dombek *et al.*, 2000; van Belkum *et al.*, 1996), allowing the differentiation of taxa by targeting highly conserved repetitive DNA elements. One colony (designated here strain 7-10-1-b^T), from a 1 : 10 dilution plated on Schaedler basal agar (Oxoid) supplemented with 5 % defibrinated horse blood, exhibited a unique BOX-PCR profile (data not shown) and was subjected to further investigation using a combination of phenotypic, genotypic and chemotaxonomic methods.

Unless specified otherwise, strain 7-10-1-b^T was cultivated in pre-reduced brain heart infusion (BHI) medium (Oxoid) supplemented with 1 % arginine hydrochloride (w/v) in Hungate tubes.

Subcultures of strain 7-10-1-b^T occurred as small, pale-white, semi-translucent colonies after 48–72 h of incubation at 37 °C under strictly anaerobic conditions on agar plates. Growth in liquid media was generally slow, with cells growing to low optical densities (data not shown). Growth could be enhanced with the addition of 1 % arginine hydrochloride (w/v) (data not shown), which is consistent with the growth requirements of other anaerobic strains isolated from the human colon (Jin *et al.*, 2007). Uematsu *et al.* (2006) reported on the metabolism of arginine by members of the genera *Slackia* and *Cryptobacterium*.

Microscopic and ultrastructural analysis

For phase-contrast microscopy, mid-exponential and stationary phase cells were visualized under phase-contrast microscopy (Zeiss). Broth cultures of strain 7-10-1-b^T revealed short-rod/coccobacillus-shaped, motile, non-spore-forming small cells (data not shown).

For electron microscopy, mid-exponential phase cells grown in BHI with and without 1 % arginine hydrochloride were prepared either as whole mounts or as embedded samples and were analysed by energy-filtered transmission electron microscopy as described by Yakimov *et al.* (1998) and Golyshina *et al.* (2000).

At the ultrastructural level, as revealed from whole-mount shadow-cast (Fig. 1a, b) and ultrathin-sectioned (Fig. 1c, d) samples, cells appeared rather unusual in morphology. In whole-mount samples, cells grown in BHI appeared flagellated (Fig. 1a; fl) with subpolar flagellum insertion. These short-rod/coccoid cells produced a small amount of slime, which may be seen as a tail-like feature at the cell end. Despite the presence of this slime, the cell wall is clearly outlined (Fig. 1a; cw). Cells grown in BHI supplemented with 1 % arginine hydrochloride, however, were surrounded/encapsulated by a thick amorphous layer of slime, seen as a smooth greyish halo around individual cells or groups of cells (Fig. 1b; sl). These samples also showed cells to have an electron-translucent, mass-deficient centre, apparently equivalent to the bacterial chromosome. The mean cell length was 1.01 ± 0.21 µm (n=35; min=0.64 µm; max=1.46 µm; median=1.01 µm) and the mean cell diameter was 509 ± 55 nm (n=49; min=403 nm; max=644 nm; median=507 nm). A characteristic feature of whole-mount or ultrathin-sectioned cells was a marked longitudinal asymmetry, with one end of the cell exhibiting a pronounced conical form (Fig. 1a–c; ae). In ultrathin-sectioned cells, the condensed chromosomal DNA is seen in the centre as electron-translucent areas (Fig. 1c, d), surrounded by a rather densely packed cytoplasm. Occasionally, bright inclusions (Fig. 1c; incl) were apparent, probably indicating low-level carbon-storage activity, perhaps as alkanoates, under the growth conditions employed. At higher magnification, the cell-wall architecture is typical of a classical Gram-positive cell wall. An outer murein layer of mean thickness 12.8 ± 0.9 nm (n=25; min=10.8 nm; max=14.7 nm; median=12.8 nm) was in direct contact with the cytoplasmic membrane (mean thickness=7.6 ± 1.1 nm; n=43; min=4.6 nm; max=9.6 nm; median=7.7 nm).

Phenotypic characterization

The type strains of all three currently recognized species of the genus *Eggerthella* (*E. lenta* DSM 2243^T, [*Eggerthella*] *hongkongensis* DSM 16106^T and *Eggerthella sinensis* DSM

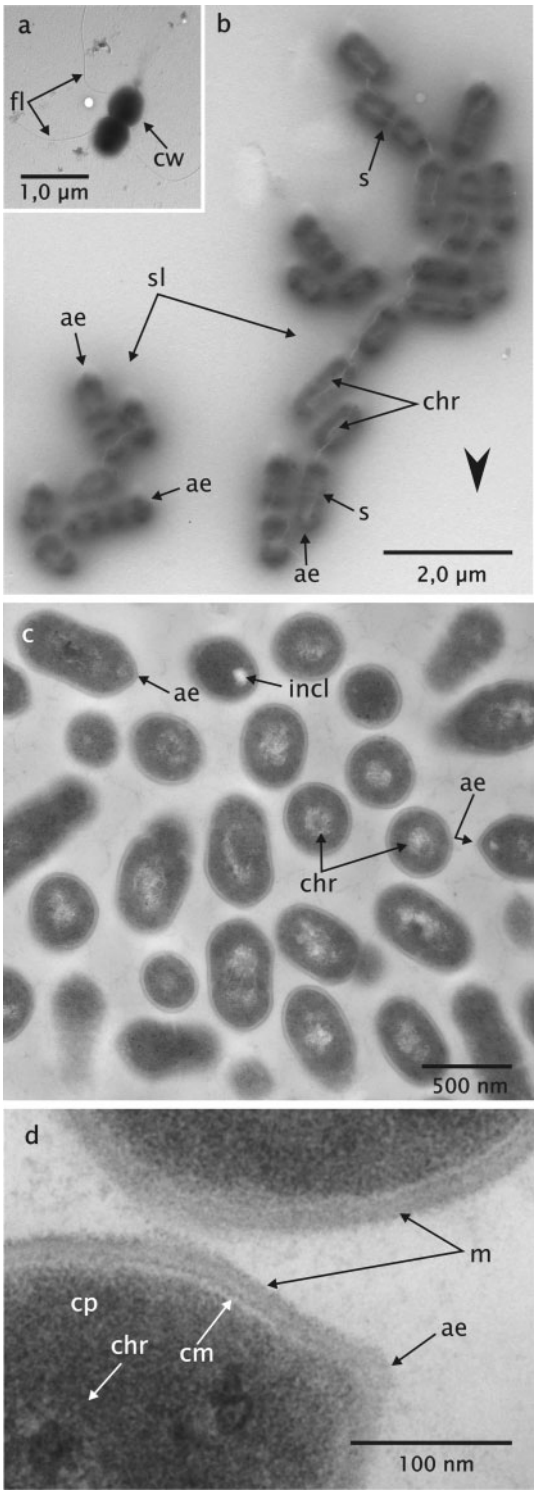

Fig. 1. Ultrastructure of cells of strain 7-10-1-b^T. (a) Whole-mount preparation of Pt-C shadow-cast cells grown in BHI. Subpolarly inserted flagella (fl) are present and the cell-wall surface (cw) can be recognized. (b) Air-dried cells grown in BHI supplemented with 1 % arginine hydrochloride are surrounded by a smooth halo of slime (sl). The chromosomal area (chr) appears translucent, surrounded by the dense cytoplasm; septum formation (s) is shown with dividing cells. The arrowhead indicates the direction of shadowing. (c, d) Ultrathin-sectioned cells, which appear translucent in the chromosomal area, surrounded by a densely packed cytoplasm. Often one cell end appears conical in shape with a pronounced apex-ending (ae); the cytoplasmic membrane (cm) is layered by a characteristic murein layer (m). incl, Cytoplasmic inclusion.

16107^T) were obtained from the DSMZ for comparative analyses and were resuscitated and maintained under the conditions specified in the DSMZ online catalogue (http://www.dsmz.de).

Routine tests (such as Gram stain and catalase activity) were carried out using standard protocols (Doetsch, 1981; McCarthy & Cross, 1984). Additional physiological tests were performed using the API Rapid ID32A and API 20A kits (bioMérieux). Conversion of a variety of different carbon sources was examined using the AN MicroPlate system (an anaerobe identification test panel) following the manufacturer's instructions (Biolog).

The biochemical characteristics of strain 7-10-1-b^T and the *Eggerthella* type strains are summarized in Table 1. Strain 7-10-1-b^T was Gram-positive and catalase-positive, but generally negative for utilization of most substrates. Only one positive reaction (arginine dihydrolase) was observed with the API kits, the only test that gave a positive reaction for all strains used in the comparison. In addition, only six carbon sources, L-methionine, L-phenylalanine, L-valine, L-valine plus L-aspartic acid, dextrin and D-glucose 6-phosphate, were metabolized in Biolog plates. Weak reactions were observed for pyruvic acid and pyruvic acid methyl ester.

Parsimony analysis of phenotypic data presented in Table 1 was performed with the program PARS (PHYLIP package, version 3.66; Felsenstein, 1989), where positive reactions were defined as 1 and negative or variable reactions declared as 0. A dendrogram representing the phenotypic differences between strain 7-10-1-b^T and the *Eggerthella* type strains was constructed (Supplementary Fig. S1, available in IJSEM Online). Three distinct branches were observed and differentiate strain 7-10-1-b^T from the members of the genus *Eggerthella*. *E. lenta* and *E. sinensis* grouped together.

Flavonoid activity

Flavonoids are polyphenolic compounds that are widely found in fruits, vegetables, nuts, seeds, flowers, tea, wine and honey, and which are ingested in significant quantities as part of the normal diet in humans (Cushnie & Lamb, 2005). Certain intestinal bacteria have a major role in the metabolism of flavonoids, which exhibit an array of health benefits to the host (Chun *et al.*, 2007).

Flavonoid conversion/degradation was tested (for strain 7-10-1-b^T only) using the fluorescence-quenching assay as

Table 1. Biochemical profiles of strain 7-10-1-b^T, [*E.*] *hongkongensis* DSM 16106^T, *E. lenta* DSM 2243^T and *E. sinensis* DSM 16107^T

Strains: 1, strain 7-10-1-b^T; 2, [*E.*] *hongkongensis* DSM 16106^T; 3, *E. lenta* DSM 2243^T; 4, *E. sinensis* DSM 16107^T. Cells of all strains are coccobacilli with a Gram-positive cell-wall architecture and staining reaction. All strains are positive for arginine dihydrolase and negative for alkaline phosphatase activity (data for *E. lenta* from Lau *et al.*, 2004b). +, Positive; −, negative; V, variable. Data are from the present study unless indicated.

Characteristic	1	2	3	4
Catalase	+	+	V[a]*	+
Metabolism of:				
β-Cyclodextrin	−	−	−	+
Dextrin	+	−	−	−
L-Fucose	−	−	−	+
D-Glucose 6-phosphate	+	−	−	−
D-Mannose	−	−	+	−
3-Methyl D-glucose	−	+	−	−
Palatinose	−	+	−	−
Raffinose	−	−	+	−
L-Rhamnose	−	+	−	−
Trehalose	−	−	+	−
Acetic acid	−	−	−	+
α-Ketobutyric acid	−	−	−	+
α-Ketovaleric acid	−	−	−	+
Pyruvic acid	+	−	−	−
Pyruvic acid methyl ester	+	−	−	−
Urocanic acid	−	+	−	+
L-Alanyl L-histidine	−	−	V	−
L-Methionine	+	+	−	−
L-Phenylalanine	+	−	−	+
L-Threonine	−	+	V	−
L-Valine	+	+	−	−
L-Valine plus L-aspartic acid	+	+	−	−
2′-Deoxyadenosine	−	−	−	+
Uridine 5′-monophosphate	−	+	−	−
Reduction of nitrate	−	−	V[a]	−
Activity of :				
α-Fucosidase	−	−	V[a]	−
β-Glucosidase	−	−†	−[a]	−
Alanine arylamidase	−	−	V[a]	−
Arginine arylamidase	−	−	V[a]	+
Glycine arylamidase	−	−	V[a]	−
Histidine arylamidase	−	−	V[a]	−
Leucine arylamidase	−	−	V[a]	−
Phenylalanine arylamidase	−	−	V[a]	−
Proline arylamidase	−	−	V[a]	−
Serine arylamidase	−	−	V[a]	−
Tyrosine arylamidase	−	−	V[b]	−
DNA G+C content (mol%)	66.4	61.1[c], 61.8	62.0, 63.8[c]	64.9[c], 65.6

*Data from: *a*, Lau *et al.* (2004b), *b*, Kageyama *et al.* (1999b); *c*, Maruo *et al.* (2008). These studies also included data from other strains.
†Different from result reported by Lau *et al.* (2004b).

described by Schoefer *et al.* (2001) although using Schaedler anaerobic agar (Oxoid) as the basal medium. In brief, nylon membranes were soaked with individual flavonoids [quercetin (10 mM), rutin (10 mM), genistein (25 mM) or phloretin (25 mM)] and a fluorescent dye, DPH (1, 6-diphenyl-1,3,5-hexatriene), and placed onto the

surface of the basal medium. Strain 7-10-1-b^T was subsequently inoculated onto the surface of the membranes and incubated under anaerobic conditions for 48–72 h at 37 °C. Plates were subsequently checked for fluorescence using a UV lamp. No significant conversion/degradation of quercetin, rutin, genistein or phloretin was observed for strain 7-10-1-b^T.

Molecular phylogenetic analysis

For 16S rRNA gene sequence determination and phylogenetic analysis, one colony was picked from a plate culture for DNA preparation, suspended in 60 µl TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) and boiled at 95 °C for 5 min and the lysate was centrifuged briefly. A 1 µl aliquot of the supernatant was used for PCR (Mullis & Faloona, 1987) targeting the 16S rRNA gene with primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) (Lane, 1991). Direct sequence determination of the PCR-amplified DNA was carried out using an ABI3130xl DNA sequencer and *Taq* cycle-sequencing reactions according to the manufacturer's instructions (Applied Biosystems). Sequences were compared initially with the EMBL nucleotide sequence database (Kanz *et al.*, 2005) using the BLAST tool (Altschul *et al.*, 1990) and were subsequently aligned with related taxa using CLUSTAL W (Thompson *et al.*, 1994). Phylogenetic relationships were estimated for evolutionarily conserved regions in MEGA (Tamura *et al.*, 2007) using the Jukes–Cantor correction model (Jukes & Cantor, 1969). A dendrogram representing evolutionary distances was generated by minimum evolution (Rzhetsky & Nei, 1992) with support for internal branches estimated from 500 bootstrap resamplings (Felsenstein, 1985). Tree topology was confirmed by maximum-likelihood using the online tool PHYML (Guindon & Gascuel, 2003; Guindon *et al.*, 2005) and was also calculated using the Jukes–Cantor model of substitution and with 500 bootstrap resamplings (Supplementary Fig. S2).

A nearly complete 16S rRNA gene sequence (1431 bp) was obtained for isolate 7-10-1-b^T which, following preliminary screening against 16S rRNA gene sequences from EMBL (Kanz *et al.*, 2005), indicated that the strain belonged to the family *Coriobacteriaceae*. The sequence was aligned with all currently accepted members of the *Coriobacteriaceae* and a phylogenetic tree representing minimum-evolutionary distances was constructed (Jukes–Cantor) with MEGA (Tamura *et al.*, 2007) (Fig. 2). On the basis of 16S rRNA gene sequence similarity, strain 7-10-1-b^T grouped with members of the genus *Eggerthella* as part of a larger assemblage that also included *Denitrobacterium detoxificans* NPOH1^T, *Adlercreutzia equolifaciens* FJC-B9^T and *Asaccharobacter celatus* do03^T. Our results indicate that the latter two taxa probably represent members of the same genus. However, whether they represent the same or different species requires further evaluation. The observed groupings were confirmed using maximum-likelihood (Supplementary Fig. S2).

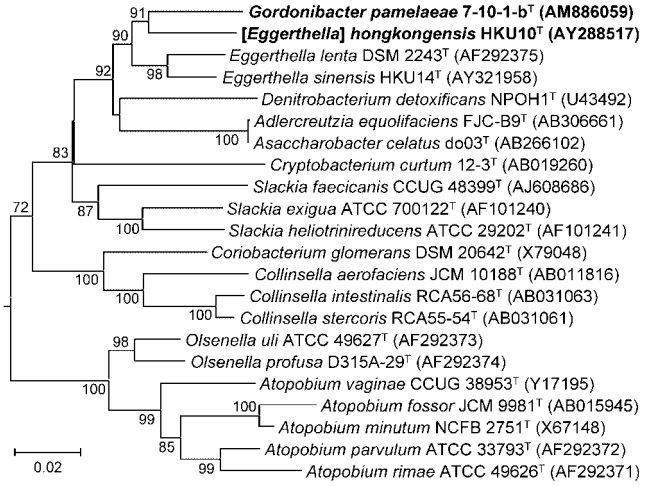

Fig. 2. Phylogenetic tree derived from minimum evolutionary distances (Jukes–Cantor) between 16S rRNA gene sequences of strain 7-10-1-b^T, the type strains of [*E.*] *hongkongensis*, *E. sinensis* and *E. lenta* and other representatives of the family *Coriobacteriaceae*. GenBank accession numbers are presented in parentheses. Bar, 2 % nucleotide sequence difference. Numbers at nodes (⩾70 %) indicate support for internal branches within the tree obtained by bootstrap analysis (percentages of 500 resamplings).

The closest relatives of strain 7-10-1-b^T are [*E.*] *hongkongensis* HKU10^T (94.6 % 16S rRNA gene sequence similarity), *E. lenta* DSM 2243^T (93.1 %) and *E. sinensis* HKU14^T (95.2 %). Strain 7-10-1-b^T formed a distinct lineage independent of the *Eggerthella* species which, also confirmed with maximum-likelihood (Supplementary Fig. S2), is evidenced by a stable branching point with high bootstrap values of 87, 91 and 94 % (percentages of 500 resamplings), respectively.

Also of interest was the relatively low 16S rRNA gene sequence similarity of [*E.*] *hongkongensis* HKU10^T to *E. sinensis* HKU14^T (95 %) and *E. lenta* DSM 2243^T (93.5 %). This was reflected in the separation of these taxa into two distinct groups and is suggestive of the fact that the genus *Eggerthella* is in need of revision. On the basis of these 16S rRNA gene sequence similarity values, DNA–DNA hybridization work was not undertaken, since the work of Nakazawa & Hoshino (2004) has shown that such low values correlate with low DNA–DNA hybridization values. Nevertheless, in consideration of the revised minimum sequence identity (94.9 ± 0.4 %) required to describe a new genus (Yarza *et al.*, 2008), it is likely that both strain 7-10-1-b^T and [*E.*] *hongkongensis* represent new genera.

G+C content of DNA

Isolation of DNA for determination of the DNA G+C content by HPLC followed described procedures (Cashion *et al.*, 1977; Mesbah *et al.*, 1989). The G+C content of the

DNA of strain 7-10-1-b^T was 66.4 mol%. This high G+C content is generally observed for the class *Actinobacteria* and is marginally higher than that observed for *E. lenta*, *E. sinensis* or [*E.*] *hongkongensis* (Table 1).

Chemotaxonomy

Fatty acids were analysed as methyl ester derivatives prepared from 10 mg dry cell material. Cells were subjected to differential hydrolysis in order to detect ester-linked and non-ester-linked (amide-bound) fatty acids (Labrenz *et al.*, 1998). Fatty acid methyl esters were analysed by gas chromatography using a 0.2 μm × 25 m non-polar capillary column and flame-ionization detection. The run conditions were: injection and detector port temperature 300 °C, inlet pressure 60 kPa, split ratio 50 : 1, injection volume 1 μl, with a temperature program from 130 to 310 °C at a rate of 4 °C min^{-1}.

Differences in fatty acid components detected by GC analysis (⩾0.5 %) were compared using the Bray–Curtis similarity algorithm (Primer 6, version 6.1.6; Primer-E). A dendrogram representing the percentage similarity in fatty acid composition between strain 7-10-1-b^T and the *Eggerthella* type strains was constructed using group average hierarchical clustering (Primer 6). The major fatty acid components that contributed to differences in fatty acid composition were assessed using the Similarity Percentages (SIMPER) tool in Primer 6. A table listing these components (⩾5 %) and their percentage composition in each of the strains was generated (Supplementary Table S1). Complete fatty acid compositions of all analysed strains are presented in Table 2.

An overall greater percentage of saturated (89 %) rather than monounsaturated (8 %) fatty acids was extracted from cells of strain 7-10-1-b^T. In comparison, cells of *E. sinensis* DSM 16107^T and *E. lenta* DSM 2243^T contained smaller amounts of saturated fatty acids (63 and 61 %, respectively) and larger quantities of monounsaturated components (33 and 36 %, respectively). Almost equal proportions of saturated and monounsaturated fatty acids were observed for [*E.*] *hongkongensis* DSM 16106^T (45 and 51 %, respectively), which was also the only strain to contain polyunsaturated fatty acids (namely 18 : 2ω6,9*c*).

Of particular interest was the relatively large percentage of branched-chain (saturated) fatty acids extracted from cells of strain 7-10-1-b^T (41 %) and their almost complete absence from [*E.*] *hongkongensis* DSM 16106^T (0.6 %) compared with *E. sinensis* DSM 16107^T (22 %) and *E. lenta* DSM 2243^T (13 %). Analysis of the major components that contributed to these differences revealed strain 7-10-1-b^T to contain larger amounts of the branched, saturated fatty acid 15 : 0 anteiso (the predominant component extracted) than obtained for [*E.*] *hongkongensis* DSM 16106^T, *E. lenta* DSM 2243^T or *E. sinensis* DSM 16107^T (Supplementary Table S1). In contrast, larger amounts of the unbranched saturated 16 : 0 dimethylacetal

Table 2. Fatty acid compositions of strain 7-10-1-b^T and type strains of [*E.*] *hongkongensis*, *E. lenta* and *E. sinensis*

Strains: 1, strain 7-10-1-b^T; 2, [*E.*] *hongkongensis* DSM 16106^T; 3, *E. lenta* DSM 2243^T; 4, *E. sinensis* DSM 16107^T. Values are percentages of the total mean amount derived from duplicate cellular fatty acid extractions. Total percentages of saturated (iso- and anteiso-branched and unbranched forms) and monounsaturated fatty acids are given in bold. Unknown fatty acids are identified by their equivalent chain length.

Fatty acid	1	2	3	4
Saturated				
Iso-branched				
13 : 0 iso	1.09	0	0	0
13 : 0 iso 3-OH	1.50	0	0	0
14 : 0 iso	6.78	0	3.91	9.25
15 : 0 iso DMA	6.07	0	0	0
15 : 0 iso	2.96	0	0.55	0
16 : 0 iso	0.66	0	0	0.65
Total iso-branched	**19.06**	**0**	**4.46**	**9.90**
Anteiso-branched				
13 : 0 anteiso	0	0	0	0.52
15 : 0 anteiso	19.79	0.63	8.50	11.43
17 : 0 anteiso DMA	1.76	0	0	0
Total anteiso-branched	**21.55**	**0.63**	**8.50**	**11.95**
(Total branched)	**(40.61)**	**(0.63)**	**(12.96)**	**(21.85)**
Unbranched				
12 : 0	2.70	4.26	1.49	0.58
14 : 0	8.71	3.78	6.51	4.15
14 : 0 DMA	9.72	0.63	1.96	2.71
16 : 0	2.42	3.92	3.50	4.12
16 : 0 DMA	13.47	23.08	29.42	22.53
16 : 0 aldehyde	0	0	0.51	0
Unknown 16.107	4.05	0	0.59	1.53
17 : 0	0.68	0	0	0
18 : 0	0.78	2.59	0.71	0.83
18 : 0 DMA	1.52	5.93	3.12	4.40
Summed feature 5*	0.52	0	0	0
Summed feature 13*	4.20	0	0	0
(Total unbranched)	**(48.77)**	**(44.19)**	**(47.81)**	**(40.85)**
Total saturated	**89.38**	**44.82**	**60.77**	**62.70**
Monounsaturated				
14 : 1ω5*c*	0	1.64	1.26	0.84
16 : 1ω7*c*	1.69	4.83	2.27	1.42
16 : 1ω7*c* DMA	0.90	1.04	1.56	1.47
18 : 1ω7*c* DMA	0	3.77	1.71	1.48
18 : 1ω9*c* DMA	1.47	6.75	5.99	7.04
18 : 1ω9*c*	3.65	30.37	22.02	19.74
Summed feature 10*	0	2.36	1.36	1.26
Total monounsaturated	**7.71**	**50.76**	**36.17**	**33.25**
Polyunsaturated				
18 : 2ω6,9*c*	0	0.78	0	0

*Summed features contain the following fatty acids: summed feature 5, 15 : 0 DMA and/or 14 : 0 3-OH; summed feature 10, 18 : 1ω7*c* and/or unknown 17.834; summed feature 13, 15 : 0 anteiso DMA and/or 14 : 0 2-OH.

(DMA) and the monounsaturated fatty acid 18 : 1ω9*c* were observed for [*E.*] *hongkongensis* DSM 16106^T, *E. lenta* DSM 2243^T and *E. sinensis* DSM 16107^T, consistent with results reported by Maruo *et al.* (2008). These differences were reflected in the hierarchical clustering of the strains and revealed the presence of at least three distinct branches representing three independent taxa (Fig. 3). A notable observation was the low similarity of strain 7-10-1-b^T to all other type strains (47 %) and the separation of [*E.*] *hongkongensis* DSM 16106^T from *E. sinensis* DSM 16107^T and *E. lenta* DSM 2243^T (74 % similarity), which clustered together (85 % similarity). This is consistent with the findings of the phenotypic and 16S rRNA gene sequence analyses of these strains.

Polar lipids and quinones

Respiratory lipoquinones and polar lipids were extracted from 100 mg freeze-dried cell material using the two-stage method described by Tindall (1990a, b). Respiratory quinones were extracted using methanol/hexane (Tindall, 1990a, b) and the polar lipids were extracted by adjusting the remaining methanol/0.3 % aqueous NaCl phase (containing the cell debris) to give a chloroform/methanol/0.3 % aqueous NaCl mixture (1 : 2 : 0.8, by vol.). The extraction solvent was stirred overnight and the cell debris was then pelleted by centrifugation. Polar lipids were recovered into the chloroform phase by adjusting the chloroform/methanol/0.3 % aqueous NaCl mixture to a ratio of 1 : 1 : 0.9 (by vol.).

Respiratory lipoquinones were separated into their different classes (menaquinones and ubiquinones) by TLC on silica gel (Macherey-Nagel art. no. 805 023), using hexane/*tert*-butylmethylether (9 : 1 v/v) as solvent. UV-absorbing bands corresponding to menaquinones or ubiquinones were removed from the plate and further analysed by HPLC. This step was carried out on an LDC Analytical HPLC (Thermo Separation Products) fitted with a reversed-phase column (Macherey-Nagel; 2 × 125 mm, 3 μm, RP_{18}) using methanol/heptane (9 : 1 v/v) as the eluent. Respiratory lipoquinones were detected by absorbance at 269 nm.

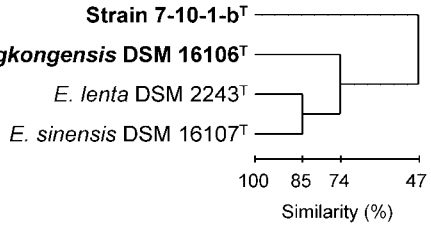

Fig. 3. Dendrogram representing the percentage similarity in cellular fatty acids between strain 7-10-1-b^T and the type strains of [*E.*] *hongkongensis*, *E. lenta* and *E. sinensis*. Values represent means of duplicate fatty acid extractions and, using group average hierarchical clustering (Bray–Curtis similarity algorithm; Primer 6), are presented as group averages.

Polar lipids were separated by two-dimensional silica gel TLC (Macherey-Nagel art. no. 818 135). The first direction was developed in chloroform/methanol/water (65 : 25 : 4, by vol.) and the second in chloroform/methanol/acetic acid/water (80 : 12 : 15 : 4, by vol.). Total lipid material and specific functional groups were detected using dodecamolybdophosphoric acid (total lipids), Zinzadze reagent (phosphate), ninhydrin (free amino groups), periodate–Schiff (α-glycols), Dragendorff reagent (quaternary nitrogen), anisaldehyde–sulphuric acid and α-naphthol (glycolipids).

The presence of DMAs in the fatty acid patterns is consistent with the presence of plasmalogens (vinyl ethers) in the polar lipids. However, it should be noted that the interpretation of the data presented here and in other publications requires some caution. While our results only indicate that DMAs are present, Maruo *et al.* (2008), Verhulst *et al.* (1987) and Anderson *et al.* (2000) also report the presence of the equivalent chain-length aldehydes. In contrast, Itoh *et al.* (1995) report only the presence of aldehydes. These differences may be due to the slightly different methods used and it should be remembered that, under certain conditions, plasmalogens hydrolyse to give first the appropriate long-chain aldehyde, which may be modified to give the equivalent DMA. The presence of aldehydes and absence of DMAs would suggest that the methods used have not converted the aldehydes to the corresponding DMA, whereas the presence of both (aldehydes and DMAs) would suggest that the conversion of the aldehydes to DMAs is not complete, while the presence of only DMAs would suggest that the reaction has gone to completion. This would need to be investigated further, but is significant in the evaluation of the data. Irrespective of which method is used, a particular aldehyde and its equivalent DMA should be treated as being derived from the same parent plasmalogen. In some cases, neither aldehydes nor DMAs are reported in members of the family *Coriobacteriaceae*, although it is not clear whether they were present, but simply not recorded in the results (Minamida *et al.*, 2008; Lawson *et al.*, 2005).

The two predominant menaquinones detected in all strains were menaquinone 6 (MK-6) and monomethylmenaquinone 6 (MMK-6) (Supplementary Table S2). Dimethylmenaquinones, in particular dimethylmenaquinone 6 (DMMK-6), were not detected in any of the strains, but this may have been due to the failure to detect this compound by HPLC, since only relatively small amounts of menaquinones were observed by TLC. DMMK-6 has been reported in a number of taxa, including members of genus *Eggerthella* (Collins *et al.*, 1985; Fernandez & Collins, 1987; Maruo *et al.*, 2008), although the relative amounts vary, suggesting both growth conditions-related variations as well as taxon-specific differences. The unidentified quinone reported in *Asaccharobacter celatus* (Minamida *et al.*, 2008) may also be DMMK-6. MK-6 was the major respiratory

lipoquinone in strain 7-10-1-b^T (59.5 %; with 40.5 % MMK-6), [*E.*] *hongkongensis* DSM 16106^T (67.8 %; with 32.2 % MMK-6) and *E. lenta* DSM 2243^T (63.7 %), whereas MMK-6 was predominant in *E. sinensis* DSM 16107^T (60.4 %) (Supplementary Table S2). These results also suggest that routine determination of the menaquinone composition within the family *Coriobacteriaceae* should not be neglected.

To our knowledge, the polar lipid composition of members of the genus *Eggerthella*, or even other taxa within the family *Coriobacteriaceae*, has not been reported previously. In all strains examined, two major phospholipids were detected, phosphatidylglycerol and diphosphatidylglycerol, as well as up to four glycolipids. All four glycolipids ran with similar, but not identical, R_f values, suggesting structural differences that may be the result of differences in the sugars present and/or the configuration of their linkages, rather than the number of sugars present. This pattern of glycolipids appears to be unique within this group of organisms. This is particularly evident given the relatively conserved polar lipid profiles of strain 7-10-1-b^T, *E. lenta* DSM 2243^T and *E. sinensis* DSM 16107^T, which were characterized by the presence of two phospholipids, phosphatidylglycerol and diphosphatidylglycerol, and four glycolipids, GL1–GL4 (Supplementary Fig. S3). [*E.*] *hongkongensis* DSM 16106^T displayed a similar pattern but lacked glycolipid GL3, further supporting its separation from the genus *Eggerthella*.

It is evident from the results presented here, which record for the first time the polar lipid, fatty acid (including plasmalogen-derived aldehydes and DMAs) and respiratory lipoquinone composition, that these three datasets are of value in the differentiation of organisms within the family *Coriobacteriaceae*. The presence of menaquinones and methylated menaquinones with six isoprenoid units is a feature that these organisms also share with certain members of the *Epsilonproteobacteria* (Carlone & Anet, 1983; Moss *et al.*, 1984; Collins *et al.*, 1984; Collins & Widdel, 1986), but they may be easily distinguished based on their fatty acid patterns. The presence of dimethylmenaquinones is, however, unique to this group of organisms. Clearly, such data may be of value in delineating higher taxa within this taxon. To date, the subclass *Coriobacteridae*, the order *Coriobacteriales* and the family *Coriobacteriaceae* are defined only in terms of 16S rRNA gene signature nucleotides (Stackebrandt *et al.*, 1997). The definition of these taxa is such that the signatures that define the subclass also define the order and family. It would seem appropriate to examine the value of chemotaxonomy in improving the definition of the order and family, or even re-examining the taxonomic infrastructure within the subclass. It is worthwhile noting that the papers of Dewhirst *et al.* (2001), Rodriguez Jovita *et al.* (1999), Collins & Wallbanks (1992), Lau *et al.* (2004b), Nakazawa *et al.* (1999), Wade *et al.* (1999), Kageyama & Benno (2000) and Kageyama *et al.* (1999a, b, c) do not include any fatty acid, polar lipid or quinone data. Such work may also have consequences for the definition of taxon-specific gene probes (Harmsen *et al.*, 2000).

In the present study, we investigated the phylogenetic, physiological/biochemical and chemotaxonomic properties of strain 7-10-1-b^T (an isolate from the colon of a patient with active Crohn's disease) in comparison with all type strains of the genus *Eggerthella*. Based on the strain's unique 16S rRNA gene sequence, its phenotypic and chemotaxonomic properties and the data obtained for its nearest neighbours, two new genera, one novel species and an emended description of the genus *Eggerthella* are proposed within the family *Coriobacteriaceae*. Thus, we propose that strain 7-10-1-b^T should be assigned as the type strain of a novel species within a new genus, for which we propose the name *Gordonibacter pamelaeae* gen. nov., sp. nov., and that [*Eggerthella*] *hongkongensis* Lau *et al.* 2006 should be transferred to a novel genus, *Paraeggerthella* gen. nov., as *Paraeggerthella hongkongensis* gen. nov., comb. nov. The characteristics that discriminate the newly proposed taxa are presented in Tables 1 and 2.

The prevalence of strain 7-10-1-b^T amongst individuals with Crohn's disease and its association with the mucosa as an opportunistic pathogen or a member of the normal flora requires further investigation.

Description of *Gordonibacter* gen. nov.

Gordonibacter (Gor.do′ni.bac′ter. N.L. masc. n. *Gordon* named after Jeffrey I. Gordon, MD, the Dr Robert J. Glaser Distinguished University Professor and Director of the Center for Genome Sciences at Washington University School of Medicine, St. Louis, MO, USA; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Gordonibacter* a rod named after Jeffrey I. Gordon).

Gram-positive, motile, non-spore-forming coccobacilli (0.5–0.6 × 0.8–1.2 μm). Obligately anaerobic. Cellular fatty acids consist mainly (~90 %) of saturated fatty acids (predominantly C_{14} and C_{15}) and include 12 : 0, 13 : 0 iso, 13 : 0 iso 3-OH, 14 : 0, 14 : 0 iso, 14 : 0 DMA, 15 : 0 iso DMA, 15 : 0 iso, 15 : 0 anteiso, 16 : 0, 16 : 0 iso, 16 : 0 DMA, 16 : 1ω7*c*, 16 : 1ω7*c* DMA, unknown ECL 16.107, 17 : 0, 17 : 0 anteiso DMA, 18 : 0, 18 : 0 DMA, 18 : 1ω9*c* DMA and 18 : 1ω9*c*; the major component is 15 : 0 anteiso. The major respiratory lipoquinone present is menaquinone MK-6; MMK-6 is a minor component. Major polar lipids are phosphatidylglycerol, diphosphatidylglycerol and four glycolipids (GL1–GL4). Oxidation/fermentation of arabinose, glucose, mannose, raffinose, trehalose and xylose is not observed. Nitrate is not reduced. The G + C content of the genomic DNA of the single known strain is 66.4 mol%. The type and only species of the genus is *Gordonibacter pamelaeae*.

Description of *Gordonibacter pamelaeae* sp. nov.

Gordonibacter pamelaeae [pa.me′la.eae. N.L. fem. n. *pamelaeae* named after Dr Pamela Lee Oxley (née Fredericks), biochemist, environmentalist, teacher, mentor and mother].

Morphology and general characteristics are as described for the genus, with cells displaying a conical cell apex. Growth is generally slow on BHI and Schaedler anaerobic media (Oxoid) supplemented with 5 % defibrinated horse blood, with pale-white, semi-translucent colonies forming after 48–72 h of incubation at 37 °C in an anaerobic environment. Growth is enhanced by the supplementation of the basal medium with 1 % arginine hydrochloride (w/v). Subpolarly inserted flagella are apparent when cells are grown in BHI medium. Cells are catalase-positive and show hydrolysis of arginine. The carbon sources L-methionine, L-phenylalanine, L-valine, L-valine plus L-aspartic acid, dextrin and D-glucose 6-phosphate are metabolized. Only weak conversion of pyruvic acid and pyruvic acid methyl ester is observed. All other organic substrates included in the Biolog AN MicroPlate are not metabolized. Chemotaxonomy is as given for the genus.

The type strain is 7-10-1-b^T (=DSM 19378^T =CCUG 55131^T), isolated from the sigmoid region of the colon of a patient suffering from active Crohn's disease.

Description of *Paraeggerthella* gen. nov.

Paraeggerthella (Pa′ra.eg.ger.thel′la. L. prep. *para* beside; N.L. fem. n. *Eggerthella* a bacterial genus name; N.L. fem. n. *Paraeggerthella* beside *Eggerthella*, named in recognition of the close relationship to the genus *Eggerthella*).

Characteristics of the genus are as described previously for [*Eggerthella*] *hongkongensis* (Lau *et al.*, 2004b; Maruo *et al.*, 2008). In addition, cellular fatty acids consist of saturated and monounsaturated fatty acids (45 : 51 in the type strain of the type species) (predominantly C_{16} and C_{18}) and include 12 : 0, 14 : 0, 14 : 0 DMA, 14 : 1ω5*c*, 15 : 0 anteiso, 16 : 0, 16 : 0 DMA, 16 : 1ω7*c*, 16 : 1ω7*c* DMA, 18 : 0, 18 : 0 DMA, 18 : 1ω7*c* DMA, 18 : 1ω9*c* DMA, 18 : 1ω9*c* and 18 : 2ω6,9*c*; the major component is 18 : 1ω9*c*. The major respiratory lipoquinone present is menaquinone MK-6; MMK-6 is a minor component. Polar lipids consist of phosphatidylglycerol, diphosphatidylglycerol and three glycolipids (GL1, GL2 and GL4). Oxidation/fermentation of arabinose, glucose, mannose, raffinose, trehalose and xylose is not observed. The G+C content of the genomic DNA of the only known strain is 61.1–61.8 mol%. The type and only species of the genus is *Paraeggerthella hongkongensis*.

Description of *Paraeggerthella hongkongensis* (Lau *et al.* 2006) comb. nov.

Basonym: *Eggerthella hongkongensis* Lau *et al.* 2006.

Characteristics of the species are as described previously by Lau *et al.* (2004b) and Maruo *et al.* (2008). In additional, physiological testing using the two API kits Rapid ID32A and API 20A reveals just one positive reaction, for arginine dihydrolase. Results obtained with Biolog AN MicroPlates indicate that the following substrates are metabolized: 3-methyl D-glucose, palatinose, urocanic acid, L-methionine, L-threonine, L-valine, L-valine plus L-aspartic acid and uridine 5′-monophosphate. Additionally, weak conversion of rhamnose is observed. The other organic substrates included in the Biolog AN MicroPlate are not metabolized. Chemotaxonomy is as given for the genus.

The type strain is HKU10^T (=DSM 16106^T =CCUG 49250^T).

Emended description of the genus *Eggerthella* Wade *et al.* 1999

When Wade *et al.* (1999) transferred *Eubacterium lentum* to the genus *Eggerthella*, the authors referred to the description of *Eubacterium lentum* published by Moore *et al.* (1971). However, the Approved Lists of Bacterial Names (Skerman *et al.*, 1980, 1989) refer to Holdeman *et al.* (1977) as the source of the description for *Eubacterium lentum*. In emending the description of the genus *Eggerthella* Wade *et al.* 1999, Maruo *et al.* (2008) also make reference to the publication of Moore *et al.* (1971) and not to Holdeman *et al.* (1977). It is important to remember that, when a name was included on the Approved Lists of Bacterial Names (Skerman *et al.*, 1980, 1989), the type of the name and the description which accompanies the name are those referenced on the Approved Lists of Bacterial Names.

The description is as given previously (Moore *et al.*, 1971; Holdeman *et al.*, 1977; Kageyama *et al.*, 1999b; Wade *et al.*, 1999; Maruo *et al.*, 2008) with the following modifications. Respiratory lipoquinones consist of MK-6 and MMK-6; MK-6 is the major component in *E. lenta* and MMK-6 in *E. sinensis*. Polar lipids consist of two phospholipids, phosphatidylglycerol and diphosphatidylglycerol, and four glycolipids, GL1–GL4. The G+C content of the genomic DNA of the known species is 62.0–63.8 mol% (*E. lenta*) and 64.9–65.6 mol% (*E. sinensis*). The genus comprises two known species, *Eggerthella lenta* (the type species) and *E. sinensis*.

Acknowledgements

We gratefully acknowledge Dr Peter Schumann for conducting the G+C analyses. We also thank Macarena Marin, Eva Medina and Ed Moore for their valuable intellectual input. This work was supported by a National Genome Research Network (NGFN II) grant (NIE-S06T14).

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