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TAXONOMIC DESCRIPTION

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Capnocytophaga periodontitidis sp. nov., isolated from subgingival plaque of periodontitis patient

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Abstract

Two carbon dioxide-requiring, gliding, Gram-stain-negative strains, designated p1a2^T and 051621, were isolated from subgingival plaque in association with severe periodontitis. The 16S rRNA gene sequence analysis revealed that they represented members of the genus *Capnocytophaga* and had less than 96.4% pairwise similarity with species with validly published names in this genus. The whole-genome sequences of those strains had less than 91.9% average nucleotide identity and 48.4% digital DNA–DNA hybridization values with the other type strains of species of the genus *Capnocytophaga*, both below the species delineation threshold. The results of pan-genomic analysis indicated that p1a2^T and 051621 shared 765 core gene families with the other ten species in this genus, and the numbers of strain-specific gene families were 493 and 455, respectively. The major fatty acids were iso-$C_{15:0}$ and $C_{16:0}$. A combination of phenotypic, chemotaxonomic, phylogenetic and genotypic data clearly indicate that p1a2^T and 051621 should be considered to represent a novel species of the genus *Capnocytophaga*, for which the name *Capnocytophaga periodontitidis* sp. nov. is proposed. The type strain is p1a2^T (=CGMCC 1.17337^T=JCM 34126^T).

The genus *Capnocytophaga* belonging to the family *Flavobacteriaceae*, class *Flavobacteria,* includes capnophilic, facultatively anaerobic, Gram-negative bacteria exhibiting gliding motility. Up to the time of writing, ten species with validly published names have been described (https://lpsn.dsmz.de/genus/capnocytophaga), among which six species were isolated from human oral cavity (*Capnocytophaga gingivalis*, *Capnocytophaga granulosa*, *Capnocytophaga* haemolytica, *Capnocytophaga leadbetteri, Capnocytophaga ochracea* and *Capnocytophaga sputigena*) [1–5], and the remaining four species inhabit dog and cat: *Capnocytophaga canimorsus*, *Capnocytophaga cynodegmi* [6], *Capnocytophaga canis* [7] and *Capnocytophaga felis* [8]. Some species of the genus *Capnocytophaga* are considered to be commensal of the human oral cavity and can be opportunistic pathogens associated with periodontal diseases, and causing systemic infection in both immunocompetent and immunocompromised hosts [9–12]. Mills *et al.* described a case of bacteraemia and possible endocarditis caused by a recently discovered genotype of *Capnocytophaga*, *Capnocytophaga* genomospecies AHN8471 [13], which may be the same species as our strains according to data on 16SrRNA gene similarity (>99%). Species of the genus *Capnocytophaga* infecting animals are recognized as zoonotic pathogens transmitted by dog/cat bite and cat scratch, for instance, *C. canimorsus* can cause fatal sepsis with a mortality rate of approximately 26% [14].

In this study, two strains p1a2^T and 051621 were isolated from severe diseased sites of chronic periodontitis patients. They were phenotypically, chemotaxonomically, and genetically

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Keywords: *Capnocytophaga periodontitidis* sp. nov; genotype; polyphasic taxonomy; phylogenetics; physiology; chemotaxonomy.
Abbreviations: ANI, average nucleotide identity; BHI-H, Brain Heart Infusion supplemented with 5 µg/mL hemin; BHI (Oxide), Brain Heart Infusion; CDS, coding sequence; CGMCC, China General Microbiological Culture Collection Center; dDDH, digital DNA–DNA hybridization; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen; GGD, Genome-to-Genome Distance; GGDC, Genome-to-Genome Distance Calculator; JCM, Japan Collection of Microorganisms; MALDI-TOF MS, matrix-assisted laser desorption/ionization time of flight mass spectrometry; NCTC, National Collection of Type Cultures; OAT, orthologous average nycleotide identity tool; SEM, scanning electron microscopy; TEM, transmission electron microscopy; TSA (Oxide), Trypticase agar; TSB-H, Trypticase soy broth supplemented with 5 µg/mL hemin; TSB (Oxide), Trypticase soy broth.
The GenBank/EMBL/DDBJ accession numbers for whole genome and 16S rRNA gene sequences of strains p1a2^T and 051621 are JAEFDB000000000, JAEFDC000000000, MW341441 and MW341444, respectively. Genome data for strains p1a2^T and 051621 can also be accessed in Global Catalogue of Microorganisms (GCM) with the accession numbers GCM10020098 and 10020097 (http://gcm.wdcm.org/typestrain/).
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Two supplementary tables and four supplementary figures are available with the online version of this article.

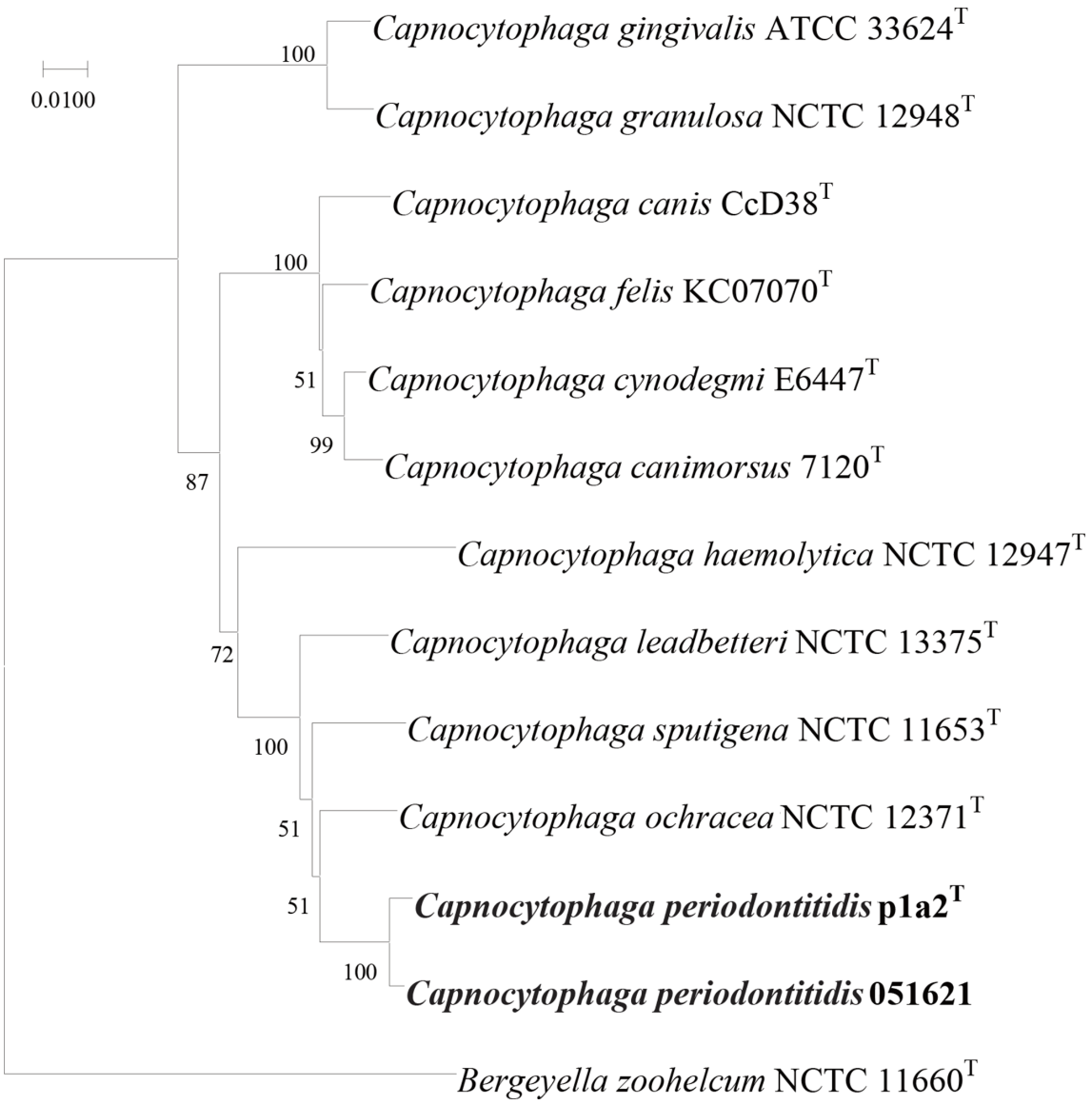

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the taxonomic relationship of strains p1a2^T and 051621 with other members of the genus *Capnocytophaga*. The phylogenetic tree was reconstructed by using the neighbour-joining algorithms. Bootstrap values (>50%) based on 500 replications are shown at the nodes of the tree. *Bergeyella zoohelcum* NCTC 11660^T served as an outgroup. Bar, 0.01 accumulated changes per nucleotide. 16S rRNA gene sequences were derived from the whole genome sequences. Extended maximum-likelihood and minimum-evolution trees are available in Figs S1 and S2. The three trees have identical topologies.

characterized and classified as representing a novel species of the genus *Capnocytophaga*.

ISOLATION AND ECOLOGY

Strain p1a2^T was isolated from subgingival plaque in a deep periodontal pocket (probing depth >6 mm) of a patient with chronic periodontitis in July 2017, and strain 051621 was isolated from subgingival plaque in a deep periodontal pocket of another patient with periodontitis in May 2017. Both strains were isolated at Peking University School and Hospital of Stomatology, Beijing, PR China. Strains were isolated by 10-fold dilution plating and incubated at 37 °C on brain–heart infusion (BHI, Oxoid) agar plates supplemented with 5% sheep blood in a CO_2 incubator (atmosphere: air +5% CO_2). The strains were stored in 20% (v/v) glycerol at −80 °C and skim milk with freeze-drying.

16S rRNA PHYLOGENY

Genomic DNA extraction and 16S rRNA gene PCR amplification and sequencing were performed as described by Cai *et al.* [15]. The similarities of 16S rRNA gene sequences between the isolates and other related species were analysed

Table 1. ANI [dDDH (%)] values between p1a2^T/051621 and the reference strains (1. *C. ochracea* NCTC12371^T; 2. *C. sputigena* NCTC 11653^T; 3. *C. leadbetteri* NCTC 13375^T; 4. *C. haemolytica* NCTC 12947^T; 5. *C. canimorsus* 7120^T; 6. *C. cynodegmi* E6447^T; 7. *C. felis* KC07070^T; 8. *C. canis* CcD38^T; 9. *C. granulosa* NCTC 12948^T; 10. *C. gingivalis* ATCC 33624^T) are shown in the lower left; whole genes and unique genes of each strain are shown in the upper and left parentheses, respectively; shared genes between any two strains are shown in the upper right

Genome	P1a2^T (2595)	051621 (2455)	1 (1888)	2 (2348)	3 (2016)	4 (1844)	5 (1711)	6 (1993)	7 (2090)	8 (1769)	9 (2105)	10 (2025)
P1a2 (493)		1840	1369	1682	1411	1283	1017	1144	1095	1080	1389	1318
051621 (455)	96.85 (69.9–75.8)		1340	1633	1365	1229	1026	1130	1161	1069	1315	1300
1 (382)	84.46 (30.5–35.5)	84.47 (30.4–35.3)		1332	1219	1210	980	1093	1095	1049	1134	1118
2 (417)	91.79 (43.2–48.4)	92.04 (43.2–48.3)	83.61 (28.7–33.6)		1421	1266	1029	1172	1207	1079	1310	1304
3 (375)	78.08 (22.9–27.7)	78.01 (22.3–27.1)	77.27 (21.4–26.1)	78.09 (22.7–27.5)		1170	1005	1083	1144	1052	1215	1161
4 (363)	71.36 (21.0–25.7)	71.31 (21.3–26.0)	72.00 (24.3–29.1)	71.23 (20.7–25.4)	71.31 (19.3–24.0)		997	1090	1116	1043	1127	1110
5 (361)	70.19 (18.9–23.6)	70.29 (19.9–24.6)	70.22 (21.1–25.9)	70.35 (21.0–25.7)	69.80 (21.4–26.2)	68.67 (22.2–27.0)		1183	1174	1156	967	966
6 (396)	70.68 (18.5–23.1)	70.75 (18.4–23.1)	70.65 (20.9–25.6)	70.82 (18.9–23.6)	70.35 (19.4–24.1)	68.87 (20.4–25.1)	79.21 (41.3–46.4)		1463	1199	1068	1072
7 (393)	70.73 (20.6–25.3)	70.47 (20.4–25.1)	70.52 (21.9–26.7)	70.88 (20.7–25.4)	70.24 (19.4–24.0)	68.98 (20.1–24.8)	75.63 (25.5030.4)	83.54 (24.4–29.3)		1198	1116	1090
8 (377)	70.44 (19.0–23.7)	70.61 (19.4–24.0)	70.45 (21.8–26.6)	70.68 (21.2–26.0)	70.30 (19.6–24.3)	68.78 (21.4–26.2)	76.35 (40.7–45.7)	77.37 (33.8–38.7)	75.77 (23.9–28.7)		1029	1010
9 (368)	71.60 (31.0–35.9)	70.60 (24.8–29.7)	70.02 (24.1–29.0)	70.77 (25.9–30.8)	70.86 (33.9–38.9)	68.25 (23.8–28.6)	67.71 (21.2–25.9)	67.48 (19.7–24.4)	67.47 (18.5–23.1)	67.42 (21.6–26.4)		1425
10 (345)	71.78 (32.8–37.8)	71.80 (31.0–35.9)	70.50 (28.3–33.2)	72.40 (35.3–40.2)	70.51 (26.8–31.7)	68.13 (24.8–29.6)	67.99 (20.9–25.6)	67.74 (20.7–25.4)	67.72 (17.7–22.3)	67.80 (19.8–24.5)	79.09 (21.426.2)	

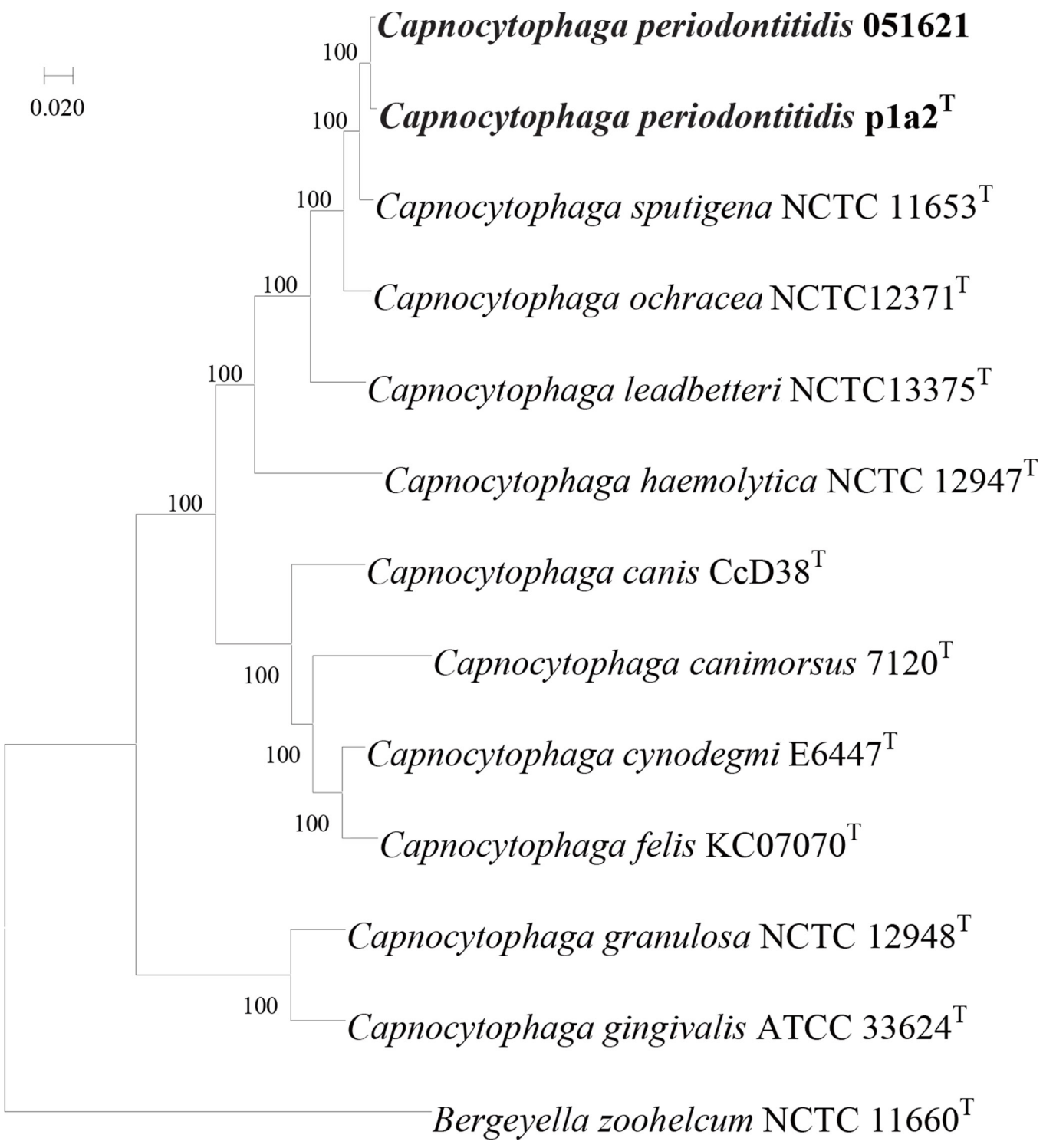

Fig. 2. Phylogenetic analyses of strains p1a2^T and 051621 tree reconstructed using gene sequences of 56 marker genes. *Bergeyella zoohelcum* NCTC 11660^T served as an outgroup. Bar, 0.02 accumulated changes per nucleotide.

using the EzTaxon server (http://eztaxon-e.ezbiocloud.net/) [16]. Multiple sequence alignments were performed using CLUSTAL W [17]. Phylogenetic analyses were performed by the neighbor-joining [18], maximum-likelihood [19] and minimum-evolution [20] methods in MEGA v 7.0 software [21] with bootstrap analysis [22] of 1000 resampled datasets.

Almost complete 16S rRNA gene sequences were determined for p1a2^T (1501 nt) and 051621 (1422 nt). They shared 99.2% pairwise similarity to each other. Furthermore, the type strain p1a2^T shared the highest pairwise similarities with *C. ochracea* NCTC 12371^T (96.1%), C. *sputigena* NCTC 11653^T (95.6%) and C. *leadbetteri* NCTC 13375^T (95.3%), with values for other species with validly published names being below 93.0%. Thus, phylogenetic trees including all species of the genus *Capnocytophaga* with validly published names were reconstructed based on 16S rRNA gene sequences. In all the phylogenetic trees reconstructed with different algorithms, the two novel isolates formed a robustly separate lineage, while clustered together with C. *sputigena* NCTC 11653^T by a large distance (Figs 1, S1 and S2, available in the online version of this article)

GENOME FEATURES

The purity and concentration of genomic DNA of strains p1a2^T and 051621 were determined using a NanoDrop 8000 spectrophotometer (Thermo Scientific). Purified genomic DNA was sheared into approximately 300–500 bp fragments and a 20 kb library was constructed according to the manufacture's protocol with T4 DNA polymerase and Klenow DNA polymerase. The paired-end library (2×150 bp) was sequenced using the HiSeq 2000 platform (Illumina). A total of 11245210 raw reads were obtained for p1a2^T, and 37382420 raw reads for 051621. The *de novo* assembly was performed by SPAdes [23]. Genome annotation was conducted by Prokka [24] and metabolic pathway-related analysis was accomplished using BlastKOALA (https://www.kegg.jp/blastkoala/). The average nucleotide identity (ANI) analysis was conducted using the orthologous average nycleotide identity tool (OAT) provided by ChunLab (https://www.ezbiocloud.net/tools/orthoani) [25]. The genome-to-genome distance (GGD) analysis was performed using the GGD calculator (GGDC) provided by the German Collection of Microorganisms and Cell Cultures (DSMZ) (http://ggdc.dsmz.de). Phylogenomic analysis based on core protein sequence comparison was achieved with OrthoMCL v1.4 (https://orthomcl.org/orthomcl/), MAFFT v7.221 (http://mafft.cbrc.jp/alignment/software/) and MEGA v 7.0 software, which uses 56 marker genes [26]. The marker genes analysis was performed using the 10 genomic sequences from species of the genus *Capnocytophaga* available on GenBank, with a genome of *Bergeyella zoohelcum* as the outgroup.

The genome of p1a2^T had 56 scaffolds with a final coverage of 500×, a total length of 3236447 bp with a N50 value of 217200, and a DNA G+C content of 38.64 mol%. The chromosome contained 3019 coding sequences (CDSs), 3 rRNA operons and 39 tRNA sequences. The genome of 051621 had 53 scaffolds with a final coverage of 500×, a total length of 3136501 bp with a N50 value of 179499, and a DNA G+C content of 38.55 mol%. The chromosome contained 2888 CDSs, 2 rRNA operons and 41 tRNA sequences. To evaluate the novel isolates, the ANI and digital DNA–DNA hybridization (dDDH) values were calculated. Strains p1a2^T and 051621 shared 96.85% ANI and 69.9–75.8% dDDH values to each other, therefore, they represent the same species but distinct clones. The values between the two novel isolates and the ten species of the genus *Capnocytophaga* with validly published names ranged from 70.2 to 92.1% and 18.4 to 48.4 %, respectively (Table 1), which are well below the thresholds of 95–96% (ANI) and 70% (dDDH) proposed for the delineation of bacterial species [19, 27]. To better characterize the status of strains p1a2^T and 051621, we have performed phylogenomic analysis based on 56 marker genes. The topology of the core genes tree was congruent with that of the tree based on 16S rRNA gene sequences and confirmed the affiliation of the newly isolated strains to the genus *Capnocytophaga* as a novel species (Fig. 2). Comparative genomics analysis was also performed to further identify traits distinctive between two isolates and the ten type strains of species of the genus *Capnocytophaga*. They shared 765 common gene families, which represent about one-third of CDS in each genome. p1a2^T and 051621 have 493 and 455 unique gene families, respectively (Table 1). Proteins encoded by genes unique to p1a2^T /051621 are mainly in the categories 'cell wall/membrane/envelope biogenesis' (47/42), 'carbohydrate transport and metabolism' (45/39), 'general function prediction only' (43/39), 'translation, ribosomal structure and biogenesis' (40/40), 'amino acid transport and metabolism' (35/33), 'replication, recombination and repair' (35/34), 'energy production and conversion' (32/31), 'inorganic ion transport and metabolism' (26/20), 'coenzyme transport and metabolism' (25/23), 'posttranslational modification, protein turnover, chaperones' (22/22), 'function unknown' (24/20), as well as 'nucleotide transport and metabolism' (19/19) (Tables S1 and S2). According to the results of the phylogenetic and phylogenomic analysis, the strains *C. sputigena* NCTC 11653^T, *C. ochracea* NCTC 12371^T, and *C. leadbetteri* NCTC 13375^T were selected as references for physiological and chemotaxonomic analysis and purchased from the NCTC.

Strain p1a2^T and 051621 showed identity to a previously described genospecies of the genus *Capnocytophaga* AHN8471 [5] and a putative novel species without a validly published name '*Capnocytophaga endodontalis*' strain ChDC OS43 [28] based on the genomes. The similarities of 16S rRNA gene sequences were 99.12–99.27% between p1a2^T and AHN8471/ChDC OS43^T, and 99.79–99.93% between 051621 and AHN8471/ChDC OS43^T. They shared 96.45–96.77% ANI and 65.6–73.6% dDDH with AHN8471/ChDC OS43^T (Table S1).

PHYSIOLOGY AND CHEMOTAXONOMY

Cell size, morphology and extracellular material were examined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). For SEM, strains were

Table 2. Physiological differences between p1a2^T and 051621, and other closely related members of genus *Capnocytophaga*: 1. *C. ochracea* NCTC 12371^T; 2. *C. sputigena* NCTC 11653^T; 3. *C. leadbetteri* NCTC 13375^T; 4. *C. gingivalis* ATCC 33624^T; 5. *C. haemolytica* ATCC 51501^T; 6. *C. granulosa* ATCC 51502^T. +, Positive; –, negative; W, weakly positive; ND: no data available; All of the strains examined in this study can produce acid from aesculin ferric citrate, and have positive enzymatic activities for L-arginine, urea and 4-nitrophenyl-βD-galactopyranoside. All of them can use maltose, cellobiose, gentiobiose, sucrose, turanose, stachyose, α-D-glucose, D-mannose, D-fructose, D-galactose, L-fucose, D-fucose, D-glucose-6-phosphate, L-glutamic acid and tween 40; and are resistant to rifamycin SV, minocycline and tetrazolium violet

Characteristic	p1a2^T	051621	1	2	3	4	5	6
Cell size (μm) width ×length	0.3–0.43×4.3–42	0.26–0.44×4.85–32.4	0.21–0.28×3.7–5.3	0.22–0.27×3.3–9.3	0.24–0.35×2.6–9.0	0.38–0.45×2.5–4.2	0.2–0.3×2.2.0–4.0	0.3–0.4×5.0–8.0
Growth conditions								
pH range (optimum)	6–9 (7.5)	6–9 (7.5)	5.5–9 (7.5)	6–9.5 (7.5)	6–9 (7.5)	ND	ND	ND
Temperature range (optimum) (°C)	25–42 (37)	32–42 (37)	25–42 (37)	32–37 (37)	25–42 (37)	30–35 (35)	ND	ND
NaCl concentration (optimum) (%)	0–1% (0)	0–1% (0)	0–1% (0)	0–1% (0)	0–1% (0)	ND	ND	ND
DNA G+C content (%)	38.64	38.55	39.59	38.44	39.80	40.50	44.20	41.50
Aesculin hydrolysis	+	+	+	+	+	–	+	–
Glucosidase activity	+	+	+	+	–	ND	ND	ND
Nitrate reduction	–	–	–	–	–	–	+	–
Acid production from								
D-galactose	+	+	–	+	–	ND	ND	ND
D-glucose	+	+	–	+	–	+	+	+
D-fructose	+	+	–	+	–	ND	ND	ND
D-mannose	+	+	–	+	W	+	+	+
Methyl α-D-mannopyranoside	–	W	–	–	–	ND	ND	ND
Methyl α-D-glucopyranoside	–	W	–	–	–	ND	ND	ND
N-acetyl-glucosamine	–	+	–	+	W	ND	ND	ND
Amygdalin	+	+	+	+	–	ND	ND	ND
Arbutin	–	W	–	–	–	ND	ND	ND
Salicin	–	W	–	–	–	ND	ND	ND
Cellobiose	–	+	–	–	–	–	–	–
Maltose	+	+	+	+	–	+	+	+
Lactose	+	+	+	+	–	+	+	+

Continued

Table 2. Continued

Characteristic	p1a2^T	051621	1	2	3	4	5	6
Melibiose	−	w	−	−	−	ND	ND	ND
Sucrose	+	+	−	+	−	ND	ND	ND
Trehalose	−	w	−	−	−	ND	ND	ND
Inulin	+	+	−	+	−	+	+	−
Melezitose	−	w	−	−	−	ND	ND	ND
Starch	+	+	−	+	+	ND	ND	ND
Xylitol	w	w	−	−	−	ND	ND	ND
Utilization of sole carbon and nitrogen source								
Dextrin	−	+	+	−	−	ND	ND	ND
Raffinose	+	+	+	−	−	ND	ND	ND
Lactose	+	+	+	+	−	ND	ND	ND
N-acetyl-D-glucosamine	+	+	+	+	−	ND	ND	ND
N-acetyl-*β*-D-mannosamine	+	+	+	+	−	ND	ND	ND
N-acetyl-D-galactosamine	+	−	−	−	−	ND	ND	ND
L-rhamnose	+	+	+	−	+	ND	ND	ND
D-serine	−	+	+	+	−	ND	ND	ND
D-fructose-6-phosphate	+	−	+	+	−	ND	ND	ND
L-aspartic acid	−	−	+	−	+	ND	ND	ND
L-histidine	−	−	+	−	−	ND	ND	ND
L-serine	−	−	−	−	+	ND	ND	ND
Pectin	−	+	+	−	−	ND	ND	ND
D-galacturonic acid	−	−	+	−	−	ND	ND	ND
L-galactonic acid lactone	−	−	+	−	−	ND	ND	ND
Glucuronamide	+	+	−	+	+	ND	ND	ND
D-lactic acid methyl ester	−	+	−	−	+	ND	ND	ND
L-lactic acid	−	+	−	−	+	ND	ND	ND
α-Ketoglutaric acid	+	−	+	+	+	ND	ND	ND

Continued

Table 2. Continued

Characteristic	p1a2^T	051621	1	2	3	4	5	6
D-malic acid	−	−	+	−	−	ND	ND	ND
L-malic acid	+	+	−	+	+	ND	ND	ND
α-hydroxybutyric acid	+	+	−	−	+	ND	ND	ND
Chemosensitivity								
Fusidic acid	+	+	−	+	−	ND	ND	ND
Troleandomycin	+	+	−	+	+	ND	ND	ND
Lincomycin	+	+	−	+	+	ND	ND	ND
Guanidine HCl	+	+	−	+	+	ND	ND	ND
Niaproof 4	−	−	−	−	+	ND	ND	ND
Tetrazolium blue	−	+	+	+	−	ND	ND	ND
Nalidixic acid	−	+	+	+	+	ND	ND	ND
Lithium chloride	+	−	−	−	−	ND	ND	ND
Potassium tellurite	−	+	+	+	+	ND	ND	ND
Aztreonam	+	+	+	+	−	ND	ND	ND
Sodium butyrate	−	+	−	+	+	ND	ND	ND
Sodium bromate	−	−	−	−	+	ND	ND	ND
Reference	This study	This study	This study	This study	This study	[2, 3, 35]	[3]	[3]

Table 3. Comparison of the cellular fatty acid content (percentages) of strains p1a2^T, 051621 and other closely related members of genus *Capnocytophaga*: 1. *C. ochracea* NCTC 12371^T; 2. *C. sputigena* NCTC 11653^T; 3. *C. leadbetteri* NCTC 13375^T; 4. *C. gingivalis* ATCC 33624^T; 5. *C. haemolytica* ATCC 51501^T; 6. *C. granulosa* ATCC 51502^T. TR: trace (less than 1%); –: not detected or not reported

Fatty acid	p1a2^T	051621	1	2	3	4	5	6
Saturated straight-chain:								
$C_{14:0}$	1.72	1.14	2.01	2.37	6.44	TR	TR	1.10
$C_{15:0}$	–	–	–	–	–	1.20	TR	TR
$C_{16:0}$	29.56	24.67	23.05	31.29	25.19	4.10	4.90	5.60
$C_{18:0}$	9.69	3.00	2.21	2.90	4.22	14.40	15.30	10.00
Unsaturated straight-chain:								
$C_{18:1}\omega 9c$	4.65	3.49	5.64	6.00	6.38	–	–	–
Saturated branched-chain:								
iso-$C_{13:0}$	TR	TR	TR	1.10	2.25	3.50	TR	1.50
iso-$C_{15:0}$	35.43	44.84	43.48	33.61	38.66	68.40	69.10	72.80
iso-$C_{17:0}$	TR	TR	TR	TR	–	TR	1.60	TR
anteiso-$C_{15:0}$	1.55	1.19	1.19	1.81	0.68	–	–	–
iso-$C_{15:0}$ 3OH	1.72	2.13	1.37	1.02	3.73	–	–	–
$C_{16:0}$ 3OH-	2.19	4.72	5.49	5.35	4.57	4.40	4.90	5.40
iso-$C_{17:0}$ 3OH	6.13	9.65	9.62	7.08	4.35	–	–	–
***Summed feature:**								
5	1.44	1.33	2.22	3.15	–	–	–	–
8	1.09	–	–	–	–	–	–	–
Reference	This study	This study	This study	This study	This study	[3]	[3]	[3]

*Summed feature 5 contains $C_{18:2}\omega 6,9c$ and/or anteiso-$C_{18:0}$; Summed feature 8 contains $C_{18:1}\omega 7c$ and/or $C_{18:1}\omega 6c$.

co-cultured with silicon wafer in Trypticase Soy Broth (TSB, Oxide) medium supplemented with 5 µg ml^{-1} hemin (TSB-H) for 24 h. For TEM, strains were grown in TSB-H for 48 h, and then 20 µl bacteria fluid was applied to a carbon-coated 300-mesh copper grid and then stained with 1% phosphotungstic acid (pH 7.2). The Gram staining was determined using a Gram stain kit (Solarbio). Gliding motility was determined by the method described by Poirier *et al.* [29]. Oxidase, catalase and anaerobic nitrate reduction activities were examined as described previously [30]. Determination of biochemical characteristics, activities of other enzymes, acid production and utilization of sole carbon and nitrogen sources were performed using the API 20NE and API 50 CH kits (bioMérieux) and the Biolog GEN III MicroStation system following manufacturers' instructions.

Growth at different temperatures (4, 14, 20, 25, 32, 37, 42, 45 and 50 °C), NaCl concentrations [0–5% (w/v) at 1% intervals] and pH values (4.0–10.0 at 0.5 pH unit intervals) was measured in brain–heart infusion medium (BHI, Oxoid) supplemented with 5 µg ml^{-1} hemin (BHI-H) after 72 h of incubation in a CO_2 incubator or anaerobic air bag, since we found that BHI facilitated the growth of those strains better than TSB.

Strains p1a2^T and 051621 can grow under strict anaerobic conditions or in an atmosphere of 5–10% CO_2. Cells were Gram-stain-negative, gliding, non-spore-forming rods 4–42 µm long and 0.3–0.5 µm wide, occurring singly or in clusters (Fig. S3, Table 2). Growth of p1a2^T occurred at 25–42 °C and of 051621 at 32–42 °C, with an optimum at 37 °C, and pH ranging from 6 to 9, with optima at 7.5, respectively. The range for the presence of NaCl was 0–1% (w/v) with optimum growth at 0%. Nitrate and nitrite reduction was not detected. indole was not produced. Urease activities were positive. Strains p1a2^T and 051621 could be differentiated from *C. ochracea* NCTC 12371^T, *C. sputigena* NCTC 11653^T and *C. leadbetteri* NCTC 13375^T by their ability to produce acid from xylitol (weakly); could be differentiated from *C. gingivalis* ATCC 33624^T and *C. granulosa* ATCC 51502^T by their positive reaction for aesculin hydrolysis; and from *C. haemolytica* ATCC 51501^T by their negative reaction for nitrate reduction. Detailed physiological and biochemical characteristics of the novel isolates and differing characteristics that distinguish them from related strains are given in the species description and Table 2, respectively.

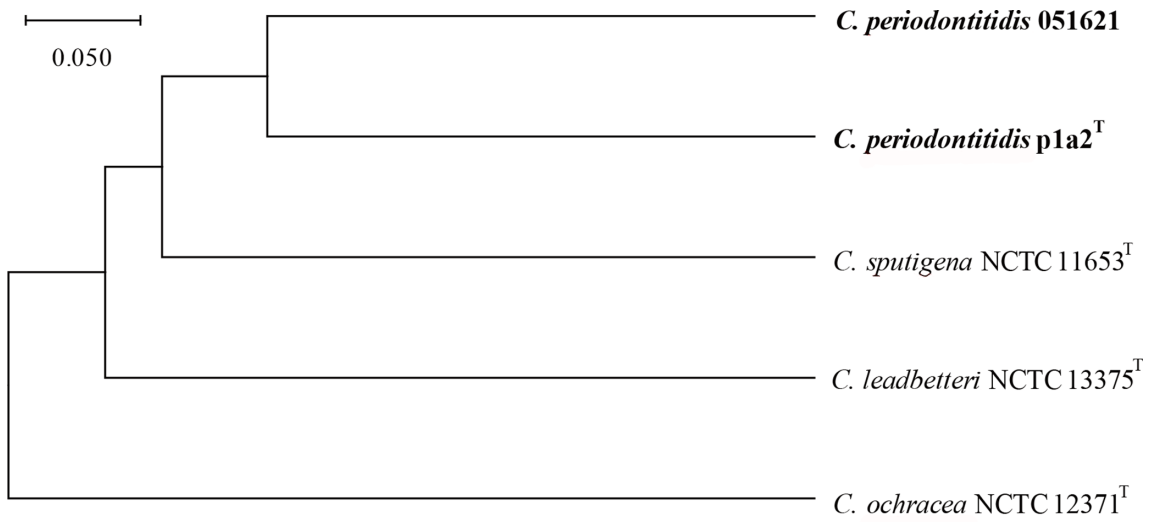

Fig. 3. Hierarchical cluster analysis based on MALDI-TOF/MS spectra.

Isolates and reference strains were incubated on trypticase soy agar (TSA, Oxoid) supplemented with 5% sheep blood at 37 °C in a CO_2 incubator and harvested when the optical density reached 70% of the maximum values. Cellular fatty acids were obtained according to the protocol of the Sherlock Microbial Identification System (MIDI), analysed by gas chromatography (model 6890; Hewlett Packard) and identified using the Microbial Identification software package version 6.0 with method TSBA6 and database TSBA6 6.00 [31]. The major cellular fatty acids analysis revealed the presence of nine fatty acids in the biomass of all tested strains of members of the genus *Capnocytophaga*. Among them, the most prevalent (percentage >3%) are the following fatty acids: iso-$C_{15:0}$, $C_{16:0}$, iso-$C_{17:0}$ 3OH, $C_{18:1}\omega9c$, and $C_{16:0}$ 3OH (Table 3). However, $C_{16:0}$ 3OH is not a major cellular fatty acid of p1a2^T, and $C_{18:0}$ is not a major cellular fatty acids of *C. ochracea* NCTC 12371^T and *C. sputigena* NCTC 11653^T as they represent less than 3%. In addition, summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) was detected in p1a2^T in appreciable amounts but not detected in any other strains. As compared with other species of the genus *Capnocytophaga* from the human oral cavity, the major cellular fatty acids of *C. gingivalis*, *C. haemolytica* and *C. granulosa*, also included iso-$C_{15:0}$, $C_{16:0}$, $C_{18:0}$, and $C_{16:0}$ 3OH, however, iso-$C_{17:0}$ 3OH and $C_{18:1}\omega9c$ were not mentioned [3].

Cells of the isolates and reference strains were incubated in BHI-H broth supplemented with 5 µg ml^{-1} hemin at 37 °C. Whole-cell protein profiles were analysed using matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry with a microTyper MS (Tianrui) as described by Zhang *et al.* [32]. The spectra were exported to MT Master 1.0 software and IDBac (https://chasemc.github.io/IDBac/) for data processing and evaluation by cluster analysis [33]. MALDI-TOF MS spectra analysis revealed the different whole-cell protein profiles of the novel strains compared with relatives in the genus *Capnocytophaga* (Fig. S4). For instance, peaks at m/z 2704–2706, 7516–7517, 7812–7813 and 9652–9654 were observed in the novel isolates but absent in the reference strains. Additionally, hierarchical cluster analysis of these protein profiles also confirmed their separateness from other closely related species of the genus *Capnocytophaga* (Fig. 3). We further compared our result with the dendrogram generated from 53 strains of members of the genus *Capnocytophaga* (48 isolates and five reference) by Jolivet-Gougeon *et. al.* [34]. It has also been reported that the closest relatedness was between *C. sputigena* ATCC 33612^T and strains AHN8471 and ChDC OS43^T that were members of the same novel species *Capnocytophaga periodontitidis*; and that there is relatively distant relatedness between *C. sputigena* ATCC 33612^T and a *C. leadbetteri* clinical isolate and *C. ochracea* ATCC 27872^T, which were consistent with the results of this study (Fig. 3).

We have compared the physiology and chemotaxonomy characteristics among p1a2^T, 051621, *Capnocytophaga* genospecies AHN8471 [5] and *Capnocytophaga endodontalis* ChDC OS43^T [28]. The ability to produce acid from D-galactose, D-glucose and D-fructose were identified as common traits that can differentiate the entire group from *C. ochracea* and *C. leadbetteri*. As to the phenotypic variations within the group, p1a2^T and 051621 could be differentiated from AHN8471 by their ability to produce acid from inulin, and from ChDC OS43^T by their positive reaction for urease. Furthermore, p1a2^T could be differentiated from ChDC OS43^T by its negative acidification reaction of cellobiose; 051621 could be differentiated from ChDC OS43 by its ability to weakly produce acid from trehalose and melezitose. As to the difference in fatty acids, $C_{17:0}$ 3OH, one of the major fatty acids in p1a2^T and 051621, was not detected in ChDC OS43^T; while 'summed feature 11', a relatively major component in ChDC OS43^T, was not detected in either p1a2^T or 051621. We failed to compare the fatty acids between p1a2^T/051621 and AHN8471 due to the lack of reported data.

In conclusion, strains p1a2^T and 051621 represent members of the genus *Capnocytophaga*, but differ from other species with validly published names in this genus on the basis of the results of the analysis of overall genome relatedness and 16S rRNA gene similarities, as well as the observed differences in physiological characteristics, fatty acid profiles and MALDI-TOF/MS spectra. We propose to classify the strains p1a2^T and 051621 as representing a novel species of the genus *Capnocytophaga*, for which the name *Capnocytophaga periodontitidis* sp. nov. is proposed. The type strain is p1a2^T.

DESCRIPTION OF *CAPNOCYTOPHAGA PERIODONTITIDIS* SP. NOV.

Capnocytophaga periodontitidis (pe.ri.o.don.ti'ti.dis. N.L. gen. n. *periodontitidis*; of periodontitis).

Cells are facultative anaerobes (growing anaerobically or in air containing 5–10% carbon dioxide, but not in normal air), rod-shaped (approximately 4–40 µm long and 0.3–0.5 µm wide) and display gliding motility. Orange and grey colonies are observed on TSB-blood agar after incubation at 37 °C for 3 days. Growth occurs at 25–42 °C (optimally at 37 °C), pH 6–9 (optimally at pH 7.5) and with 0–1% (w/v) NaCl (optimally with 0% NaCl). The major fatty acids are iso-$C_{15:0}$ and $C_{16:0}$. Catalase and oxidase activities are negative. Acid is produced from D-galactose, D-glucose, D-fructose, D-mannose, amygdalin, aesculin ferric citrate, maltose, lactose, Sucrose, inulin, raffinose, starch, glycogen, xylitol and gentiobiose. Enzymatic activities are positive for D-glucose, L-arginine, urea and 4-nitrophenyl -*β*-D-galactopyranoside. Utilisable substrates include maltose, cellobiose, gentiobiose, sucrose, turanose, stachyose, raffinose, α-D-lactose, α-D-glucose, *N*-acetyl-D-glucosamine, *N*-acetyl-ß-D-mannosamine, D-mannose, D-fructose, D-galactose, L-fucose, D-fucose, L-rhamnose, D-glucose-6-phosphate, L-glutamic acid, glucuronamide, L-malic acid, tween 40 and α-hydroxybutyric acid; resistant to 1% sodium lactate, fusidic acid, troleandomycin, lincomycin, guanidine HCl, rifamycin SV, minocycline, aztreonam, and tetrazolium violet; and sensitive to niaproof 4 and sodium bromate.

Strains of this species, p1a2^T and 051621, were recovered from subgingival plaque samples of two different periodontitis patients at Peking University School and Hospital of Stomatology, Beijing, PR China during May to July, 2017. The type strain is p1a2^T. The DNA G+C content is 38.5–38.6 mol%. They have been deposited in the China General Microbiological Culture Collection Centre as CGMCC 1.17337^T and 1.17505, and in the Japan Collection of Microorganisms for type cultures as JCM 34126^T and JCM 34128.

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Author contributions
Y.Z., conceptualization, data curation, investigation, resources, writing original draft. D.Q., data curation, investigation. W.S., formal analysis, funding acquisition. D.W., investigation. M.C., conceptualization, validation, visualization, review and editing draft.

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

Ethical statement
The study protocol was approved by the institutional review board of Peking University School and Hospital of Stomatology (Beijing, PR China) (approval number: PKUSSIRB-201522050); informed consent was obtained from the subjects.

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