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TAXONOMIC DESCRIPTION

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# *Campylobacter blaseri* sp. nov., isolated from common seals (*Phoca vitulina*)

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**Abstract**

During a study to assess the faecal microbiome of common seals (*Phoca vitulina*) in a Dutch seal rehabilitation centre, 16S rRNA gene sequences of an unknown *Campylobacter* taxon were identified. *Campylobacter* isolates, which differed from the established *Campylobacter* taxa, were cultured and their taxonomic position was determined by a polyphasic study based on ten isolates. The isolates were characterized by 16S rRNA and *atpA* gene sequence analyses and by conventional phenotypic testing. Based on the whole genome sequences, the average nucleotide identity and core genome phylogeny were determined. The isolates formed a separate phylogenetic clade, divergent from all other *Campylobacter* taxa and most closely related to *Campylobacter corcagiensis*, *Campylobacter geochelonis* and *Campylobacter ureolyticus*. The isolates can be distinguished phenotypically from all other *Campylobacter* taxa based on their lack of motility, growth at 25 °C and growth on MacConkey agar. This study shows that these isolates represent a novel species within the genus *Campylobacter*, for which the name *Campylobacter blaseri* sp. nov. is proposed. The type strain for this novel species is 17S00004-5$^T$ (=LMG 30333$^T$=CCUG 71276$^T$).

A growing number of *Campylobacter* species have been isolated from marine mammals [1–3]. The species *Campylobacter insulaenigrae* and *Campylobacter pinnipediorum* have been shown to be associated with pinnipeds [1, 3]. These species belong to separate divergent clades within the *Campylobacter* genus; *C. insulaenigrae* belongs to the clade which includes the clinically relevant species *Campylobacter jejuni*, while *C. pinnipediorum* belongs to the clade which includes *Campylobacter concisus*. Both *Campylobacter* species have also been isolated from the widely distributed common seal (*Phoca vitulina*), suggesting that this species serves as an important host reservoir for *Campylobacter*. Furthermore, seals may harbour a larger *Campylobacter* diversity than currently known, which is supported by microbiome data [4]. Here we describe a novel, urease-positive, non-motile *Campylobacter* species isolated from common seals, which is most closely related to *Campylobacter corcagiensis*, *Campylobacter geochelonis* and *Campylobacter ureolyticus*.

During a study to assess the faecal microbiome of common seals in a Dutch seal rehabilitation centre, partial 16S rRNA gene sequences (450 bp of the V3 and V4 regions) of an unknown *Campylobacter* taxon were obtained, which showed 95 % BLAST identity to *C. jejuni*, *C. corcagiensis* and *Campylobacter sputorum* (Rubio-García, unpublished work). Noteworthy, this *Campylobacter* taxon was the tenth most common species observed in the seal microbiome and comprised up to 20 % of all sequences in some seals, with loads increasing with age (3–6 months). The 16S rRNA gene sequences of this *Campylobacter* taxon were identified in 35 out of 98 animals, which were unrelated and did not have prior contact with each other, already at uptake in the rehabilitation centre, indicating that it is a widespread microbiome constituent of wild common seals outside a captive setting as well.

In an attempt to isolate this *Campylobacter* taxon for further characterization, faecal swab samples were collected in 2017

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**Keywords:** *Campylobacter*; novel species; common seal; microbiome; core genome phylogeny; average nucleotide identity.

**Abbreviations:** ANI, average nucleotide identity; CCDA, charcoal cefoperazone deoxycholate agar; DDH, DNA–DNA hybridization; TSI agar, triple sugar iron agar; TTE broth, Thomann transport and enrichment broth.

The GenBank accession numbers for the whole genome sequences of isolates 17S00004-5 and 17S00008-12 are PDHH00000000 and PDHI00000000, respectively. The GenBank accession numbers for the *atpA* sequences of isolates 17S00004-5 and 17S00008-12 are MG958595 and MG958596, respectively. The GenBank accession numbers for the 16S rRNA sequences of isolates 17S00004-5, 17S00008-12, 17S01633-4, 17S01636-3, 17S01637-3, 17S01639-3, 17S01643-3, 17S01644-3, 17S01646-3, and 17S01649-5 are MG013475, MG013476, MG013477, MG013478, MG013479, MG013480, MG013481, MG013483, MG013485 and MG013486, respectively.

One supplementary figure is available with the online version of this article.

from 40 common seals in January ($n$=20) and in September ($n$=20). Swabs were extracted in 1 ml PBS (Gibco) prior to direct culturing. Specimens from the first sampling event were cultured directly on Columbia agar with 5 % sheep blood (Oxoid) with a filter membrane (0.65 µm; Millipore) using 300 µl of the suspension, followed by incubation under aerobic conditions at 37 °C for 30 min and subsequent removal of the filter, and on CCD, Preston, and Skirrow agar (Oxoid) using 10 µl of the suspension. Samples were also cultured stagnant in three different selective pre-enrichment media (Bolton, Preston, and Thomann transport and enrichment (TTE) medium [5]; Oxoid), 14 ml of each medium in a 15 ml tube (Sarstedt) with a closed screwcap under aerobic conditions at 37 °C for 48 h, followed by incubation on Columbia agar with 5 % sheep blood with a filter membrane. All agar plates were incubated under microaerobic conditions (83.3 % $N_2$, 7.1 % $CO_2$, 3.6 % $H_2$, and 6 % $O_2$) at 37 °C for 48–72 h. Additionally, all samples were also cultured both directly and after pre-enrichment on blood agar plates with and without a filter membrane under anaerobic conditions at 37 °C for 48–72 h. Based on results from the first sampling event, samples of the second sampling event were cultured on Preston and Skirrow agar, with and without a selective pre-enrichment in Preston and TTE broth, and incubated in a microaerobic atmosphere at 37 °C for 48–72 h. For the pre-selective enrichment, samples were cultured shaking in 5 ml of each medium in a vertically positioned 25 $cm^2$ cell culture flask (Corning) with a loose screwcap under microaerobic conditions at 37 °C for 48 h. All isolates of the unknown *Campylobacter* taxon were obtained by direct culturing on Preston and Skirrow agar. In addition, *C. insulaenigrae* was isolated from one animal using the filtration method under microaerobic conditions. In total, 12 isolates of the unknown *Campylobacter* taxon were obtained from ten different animals, of which ten isolates were used for further taxonomic characterization (Table 1).

A polyphasic approach was used to determine the taxonomic position of the ten isolates [6]. Comparisons based on 16S rRNA and *atpA* gene sequences were made to determine the taxonomic position of all isolates. Whole genome sequencing was performed on two isolates on the basis of which the average nucleotide identity (ANI) and phylogenies based on the full AtpA protein and core genome could be determined. Phenotypic characteristics were determined by conventional biochemical testing for all isolates.

Complete genome sequences for isolates 17S00004-5 and 17S00008-12 were obtained by Illumina MiSeq sequencing (Illumina), with a 2×250 bp paired-end output. Sequencing reads were assembled using SPAdes version 3.1.1, resulting in 1 869 677 and 1 869 950 bp genomes, which consisted of 30 and 33 contigs for isolates 17S00004-5 and 17S00008-12, respectively. The GenBank accession numbers for the genome sequences of isolates 17S00004-5 and 17S00008-12 are PDHH00000000 and PDHI00000000, respectively (Table 1).

The taxonomic position of all isolates was determined by comparison of 16S rRNA gene sequences. The 16S rRNA gene sequences (≥1211 bp) were extracted from the whole genome sequences or obtained by PCR and Sanger sequencing [7]. Accession numbers are listed in Table 1. The 16S rRNA gene sequences of the other *Campylobacter* taxa were obtained from EzTaxon [8]. Sequence alignment and dendrogram reconstruction were performed using MEGA version 6.05 [9]. A neighbour-joining dendrogram containing all *Campylobacter* taxa was reconstructed, with bootstrap values based on 500 repetitions (Fig. 1). The 16S rRNA gene sequence of *Arcobacter butzleri* strain ATCC 49616$^T$ was used as an outgroup to root the tree. The 16S rRNA gene sequence similarity between the isolates was 99–100 %, while the sequence similarity between these isolates and the most closely related species, *C. geochelonis*, was 94 %.

For improved taxonomic resolution [10], full AtpA protein sequences were extracted from the whole genome sequences or obtained from GenBank. Alignment and dendrogram reconstruction were performed as described for 16S rRNA gene phylogeny; the AtpA sequence of *Arcobacter butzleri* strain ATCC 49616$^T$ was used as an outgroup to root the tree. Consistent with the 16S rRNA phylogeny, the isolates formed a clade distinct from other *Campylobacter* taxa, with *C. geochelonis* being the most closely related species (Fig. 2).

Additional intraspecific variation amongst all isolates was explored based on partial *atpA* sequences (489 nt), extracted from the whole genome sequences or obtained by PCR and

**Table 1.** Features of the *C. blaseri* sp. nov. isolates used in this study

| Isolate | Host species | Isolation date | 16S rRNA gene accession no. | *atpA* accession no. | Whole genome |
|---|---|---|---|---|---|
| 17S00004-5$^T$ | *Phoca vitulina* | 6 January 2017 | MG013475 | MG958595 | PDHH00000000 |
| 17S00008-12 | *Phoca vitulina* | 6 January 2017 | MG013476 | MG958596 | PDHI00000000 |
| 17S01633-4 | *Phoca vitulina* | 12 September 2017 | MG013477 | N/A | N/A |
| 17S01636-3 | *Phoca vitulina* | 11 September 2017 | MG013478 | N/A | N/A |
| 17S01637-3 | *Phoca vitulina* | 10 September 2017 | MG013479 | N/A | N/A |
| 17S01639-3 | *Phoca vitulina* | 12 September 2017 | MG013480 | N/A | N/A |
| 17S01643-3 | *Phoca vitulina* | 10 September 2017 | MG013481 | N/A | N/A |
| 17S01644-3 | *Phoca vitulina* | 10 September 2017 | MG013483 | N/A | N/A |
| 17S01646-3 | *Phoca vitulina* | 10 September 2017 | MG013485 | N/A | N/A |
| 17S01649-5 | *Phoca vitulina* | 14 September 2017 | MG013486 | N/A | N/A |

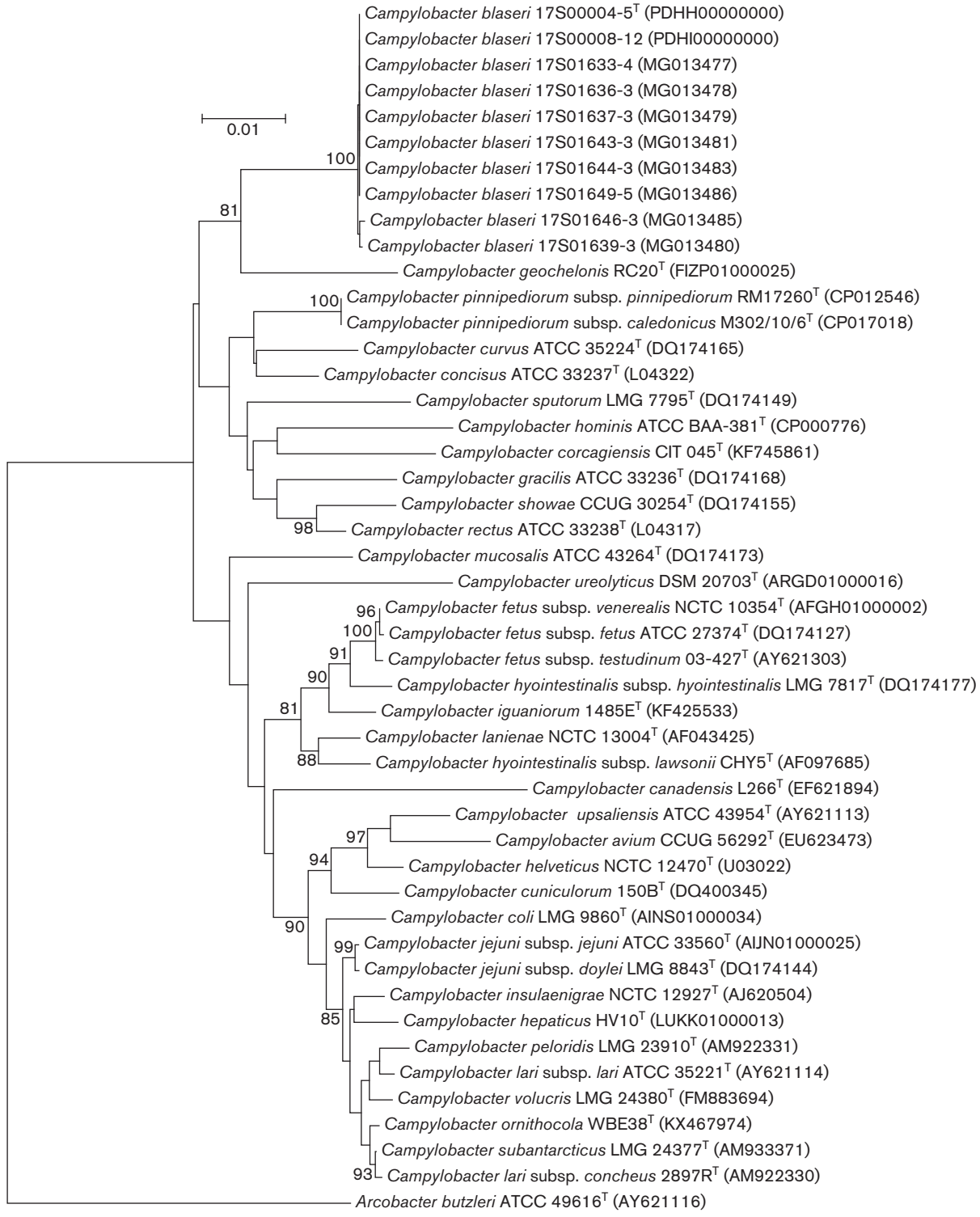


**Fig. 1.** Neighbour-joining phylogenetic dendrogram based on 16S rRNA gene sequences. *Arcobacter butzleri* ATCC 49616$^T$ is used as an outgroup and root. Bootstrap values (≥75 %) based on 500 replications are indicated at the nodes. Bar, 0.01 substitutions per nucleotide position.

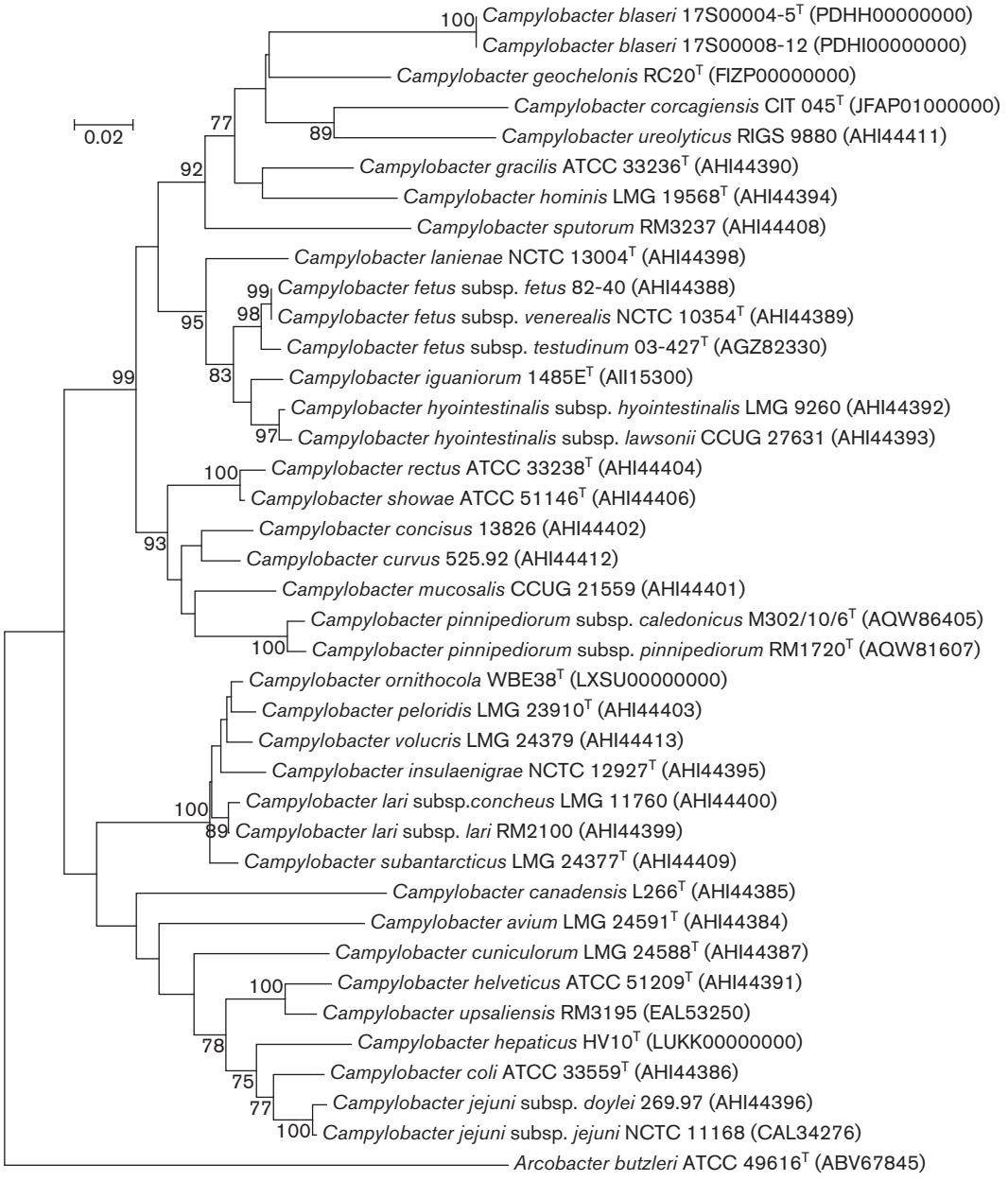


**Fig. 2.** Neighbour-joining phylogenetic dendrogram based on AtpA protein sequences. Bootstrap values (≥75 %) based on 500 replications are indicated at the nodes. *Arcobacter butzleri* ATCC 49616$^T$ is used as an outgroup and root. Bar, 0.02 substitutions per amino acid position.

Sanger sequencing. Primers and PCR conditions were as described previously [10], with primers adapted as follows: forward primer 5′ GGT CAA GAT GTT GTA TGT GTG TAT GTT GC 3′ and reverse primer 5′ TTT AAT ATT TCA ACC ATT TTT TCA CC 3′. Alignment and dendrogram reconstruction were performed as described for the 16S rRNA gene phylogeny. The intraspecific variation was similar as observed for the 16S rRNA gene phylogeny, with the exception that isolate 17S01649-5 formed an additional genetic variant (Fig. S1, available in the online version of this article).

In support of the 16S rRNA gene and AtpA phylogeny, a core genome phylogeny without correction for recombination was reconstructed using Fasttree [11], based on a 407 260 bp core gene superalignment of 382 core genes of which the protein sequences had at least 35 % sequence identity as determined by Roary [12], from genomes re-annotated using Prokka [13]. The genome of *Helicobacter pylori* strain J99 was used as an outgroup to root the tree and to find orthologs of known virulence factors using VFDB [14]. Consistent with the 16S rRNA gene phylogeny, the isolates formed a clade distinct from other *Campylobacter* taxa (Fig. 3). The most closely

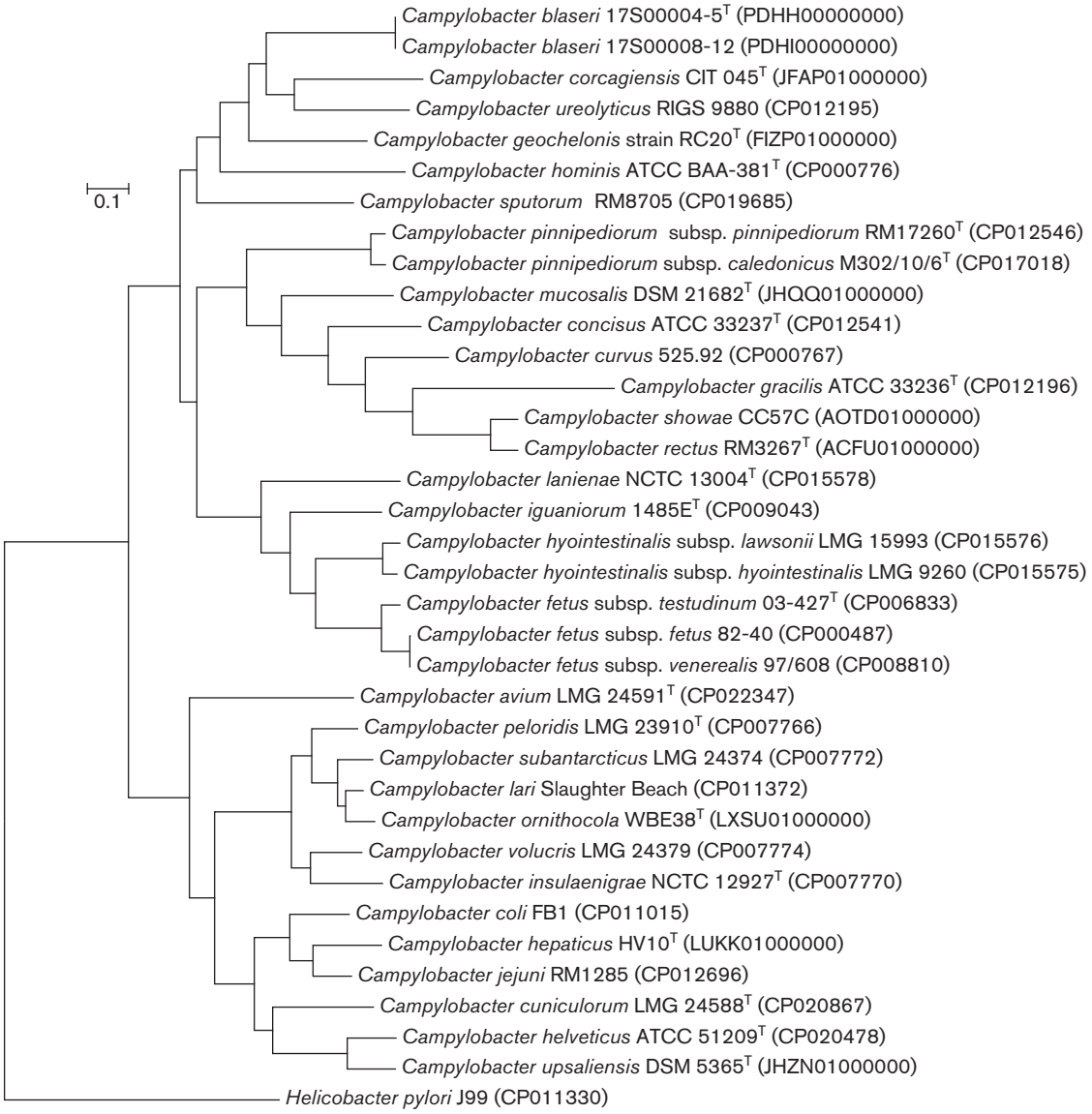


**Fig. 3.** Phylogenetic dendrogram based on the core genome. *Helicobacter pylori* J99 is used as an outgroup and root. Local support values, calculated with the Shimodaira–Hasegawa test [25], for all branches were 1. Bar, 0.1 substitutions per nucleotide position in the core gene superalignment.

related species were *C. corcagiensis*, *C. ureolyticus* and *C. geochelonis*.

Notable virulence factors, such as flagella encoding genes, *kps* genes coding for capsule production, S-layer protein encoding genes, but also the cytolethal distending toxin encoding *cdtABC* genes, which are widely conserved in *Campylobacter*, were absent from the genomes of both isolates. The majority of the virulence factors absent were related to the flagella and glycosylation of the flagella.

As an alternative for DNA–DNA hybridization (DDH), ANI has been suggested [15, 16]. A DDH species delineation of 70 % corresponds to about 95 % ANI [17]. Using JSpecies version 1.2.1 [18], pair-wise ANI values based on whole genome sequences were calculated for the seal-associated *Campylobacter* isolates and other taxa of the *Campylobacter* genus. ANI values of the genomes of the isolates were 100 % amongst each other (Table 2). The ANI between the genomes of the isolates and most closely related species *C. ureolyticus* and *C. geochelonis* was maximally 72.2 and 71.9 %, respectively, which is well below the 95 % species cut-off suggested by Goris and colleagues [17].

The G+C content was determined based on the whole genome sequences using Artemis version 13.2 [19]. Both isolates had a G+C content of 29.2 %, which is within the lower end of the range observed for *Campylobacter* (Table 3).

**Table 2.** Average nucleotide identity (ANI) values (%) based on BLAST for *C. blaseri* sp. nov. and other *Campylobacter* taxa

Strains: 1, *Campylobacter blaseri* sp. nov. 17S00004-5$^T$; 2, *C. blaseri* sp. nov. 17S00008-12; 3, *C. ureolyticus* RIGS 9880; 4, *C. corcagiensis* CIT 045$^T$; 5, *C. geochelonis* RC20$^T$; 6, *C. hominis* ATCC BAA-381$^T$; 7, *C. sputorum* RM8705; 8, *C. lanienae* NCTC 13004$^T$; 9, *C. iguaniorum* 1485E$^T$; 10, *C. hyointestinalis* subsp. *lawsonii* LMG 15993; 11, *C. hyointestinalis* subsp. *hyointestinalis* LMG 9260; 12, *C. fetus* subsp. *testudinum* 03–427; 13, *C. fetus* subsp. *fetus* 82–40; 14, *C. fetus* subsp. *venerealis* 97–608; 15, *C. pinnipediorum* subsp. *pinnipediorum* RM17260$^T$; 16, *C. pinnipediorum* subsp. *caledonicus* M302/10/6; 17, *C. mucosalis* DSM 21682$^T$; 18, *C. concisus* ATCC 33237$^T$; 19, *C. curvus* 525–92; 20, *C. gracilis* ATCC 33236$^T$; 21, *C. showae* CC57C; 22, *C. rectus* RM3267; 23, *C. upsaliensis* DSM 5365$^T$; 24, *C. helveticus* ATCC 51209$^T$; 25, *C. cuniculorum* LMG 24588$^T$; 26, *C. jejuni* RM1285; 27, *C. hepaticus* HV10$^T$; 28, *C. coli* FB1; 29, *C. ornithocola* WBE38$^T$; 30, *C. lari* Slaughter Beach; 31, *C. subantarcticus* LMG 24374; 32, *C. peloridis* LMG 23910$^T$; 33, *C. insulaenigrae* NCTC 12927$^T$; 34, *C. volucris* LMG 24379; 35, *C. avium* LMG 24591$^T$.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | --- | 100.0 | 72.1 | 71.0 | 71.9 | 68.4 | 70.4 | 68.2 | 68.2 | 68.4 | 68.6 | 68.6 | 68.5 | 68.6 | 70.7 | 69.6 | 67.4 | 67.0 | 64.8 | 64.5 | 64.8 | 64.4 | 66.3 | 66.3 | 66.5 | 67.4 | 67.5 | 67.2 | 67.9 | 68.0 | 67.6 | 68.2 | 68.0 | 68.1 | 66.4 |
| 2 | 100.0 | --- | 72.1 | 71.0 | 71.8 | 68.4 | 70.3 | 68.1 | 68.2 | 68.4 | 68.6 | 68.6 | 68.5 | 68.6 | 70.6 | 69.5 | 67.4 | 67.0 | 64.8 | 64.5 | 64.8 | 64.4 | 66.3 | 66.3 | 66.5 | 67.3 | 67.5 | 67.2 | 67.8 | 68.0 | 67.6 | 68.2 | 67.9 | 68.1 | 66.4 |
| 3 | 72.2 | 72.2 | --- | 72.5 | 71.2 | 70.2 | 70.3 | 68.0 | 68.4 | 68.4 | 68.7 | 68.6 | 68.4 | 68.5 | 68.9 | 68.8 | 67.7 | 67.2 | 65.0 | 64.8 | 65.0 | 64.7 | 66.5 | 66.6 | 66.8 | 67.8 | 67.5 | 67.4 | 68.2 | 68.1 | 68.0 | 68.3 | 68.0 | 68.4 | 66.7 |
| 4 | 70.9 | 70.9 | 72.4 | --- | 70.5 | 68.2 | 69.8 | 68.0 | 68.1 | 68.0 | 68.3 | 68.1 | 67.9 | 68.1 | 68.2 | 67.8 | 67.3 | 67.1 | 65.0 | 64.7 | 65.1 | 64.6 | 65.9 | 65.9 | 66.3 | 67.0 | 66.7 | 66.7 | 67.1 | 67.3 | 67.1 | 67.2 | 66.9 | 67.4 | 66.1 |
| 5 | 71.9 | 71.9 | 71.0 | 70.4 | --- | 68.7 | 69.7 | 68.2 | 69.2 | 68.9 | 69.0 | 69.2 | 68.8 | 68.8 | 68.5 | 68.4 | 68.0 | 68.2 | 66.4 | 66.7 | 66.5 | 66.0 | 66.4 | 66.4 | 66.0 | 66.9 | 66.4 | 66.8 | 67.0 | 67.2 | 66.8 | 67.2 | 66.5 | 67.1 | 66.0 |
| 6 | 68.6 | 68.6 | 70.2 | 68.3 | 69.0 | --- | 69.0 | 66.9 | 67.7 | 67.9 | 67.9 | 68.0 | 67.9 | 67.9 | 67.6 | 67.6 | 67.0 | 66.9 | 65.6 | 66.9 | 65.9 | 65.6 | 66.2 | 66.0 | 66.3 | 67.1 | 66.9 | 67.0 | 67.0 | 66.8 | 66.9 | 66.9 | 66.7 | 67.1 | 65.7 |
| 7 | 70.3 | 70.3 | 70.3 | 69.8 | 69.6 | 68.9 | --- | 67.9 | 68.2 | 68.5 | 68.8 | 68.8 | 68.7 | 68.7 | 69.1 | 69.1 | 67.6 | 67.2 | 65.1 | 64.6 | 65.0 | 64.6 | 66.4 | 66.4 | 66.9 | 67.5 | 67.6 | 67.3 | 68.0 | 67.9 | 67.5 | 68.2 | 67.8 | 68.2 | 66.5 |
| 8 | 68.2 | 68.2 | 68.1 | 68.1 | 68.5 | 66.9 | 68.2 | --- | 71.5 | 71.9 | 73.9 | 72.0 | 71.7 | 71.8 | 67.9 | 68.0 | 68.0 | 67.6 | 66.2 | 65.9 | 66.2 | 65.9 | 66.5 | 66.6 | 66.5 | 67.1 | 67.0 | 67.3 | 67.3 | 67.3 | 67.2 | 67.5 | 67.3 | 67.6 | 66.6 |
| 9 | 68.6 | 68.6 | 68.4 | 68.4 | 69.8 | 67.8 | 68.6 | 71.6 | --- | 75.4 | 75.5 | 75.5 | 75.0 | 75.0 | 68.4 | 68.4 | 68.2 | 68.5 | 67.1 | 66.5 | 67.2 | 66.9 | 66.1 | 66.1 | 66.1 | 67.1 | 66.7 | 67.0 | 67.1 | 67.3 | 67.1 | 67.3 | 66.7 | 67.0 | 66.2 |
| 10 | 68.5 | 68.5 | 68.5 | 68.1 | 69.2 | 67.9 | 68.6 | 71.9 | 75.4 | --- | 94.4 | 79.2 | 78.0 | 78.0 | 68.4 | 68.4 | 67.9 | 68.1 | 66.6 | 66.1 | 66.9 | 66.5 | 66.0 | 66.3 | 66.1 | 67.1 | 66.7 | 66.9 | 67.0 | 67.2 | 66.9 | 67.3 | 66.9 | 67.2 | 66.4 |
| 11 | 68.9 | 68.9 | 68.9 | 68.5 | 69.3 | 67.9 | 69.1 | 73.8 | 75.5 | 94.3 | --- | 78.2 | 77.3 | 77.4 | 68.7 | 68.6 | 68.0 | 68.2 | 66.8 | 66.1 | 66.8 | 66.4 | 66.6 | 66.5 | 66.4 | 67.3 | 67.1 | 67.3 | 67.5 | 67.6 | 67.2 | 67.7 | 67.3 | 67.6 | 66.4 |
| 12 | 68.7 | 68.7 | 68.6 | 68.2 | 69.3 | 67.7 | 69.1 | 71.9 | 75.4 | 79.2 | 78.0 | --- | 91.9 | 92.0 | 68.8 | 68.6 | 67.9 | 67.8 | 66.4 | 65.7 | 66.6 | 66.1 | 66.1 | 66.3 | 66.1 | 67.0 | 66.9 | 67.0 | 67.3 | 67.2 | 67.1 | 67.3 | 67.0 | 67.3 | 66.2 |
| 13 | 68.5 | 68.5 | 68.4 | 68.0 | 68.8 | 67.7 | 68.7 | 71.8 | 74.9 | 77.9 | 77.2 | 92.0 | --- | 99.7 | 68.3 | 68.3 | 67.6 | 67.5 | 66.5 | 65.7 | 66.4 | 66.1 | 66.0 | 66.1 | 66.2 | 66.9 | 66.7 | 66.9 | 67.3 | 67.2 | 67.1 | 67.3 | 66.9 | 67.2 | 66.3 |
| 14 | 68.9 | 68.9 | 68.7 | 68.2 | 69.1 | 68.1 | 69.0 | 71.9 | 75.0 | 78.2 | 77.4 | 92.1 | 99.6 | --- | 68.7 | 68.5 | 67.6 | 67.5 | 66.4 | 65.8 | 66.6 | 66.3 | 66.0 | 66.2 | 66.3 | 67.0 | 66.8 | 67.1 | 67.3 | 67.3 | 67.2 | 67.4 | 66.9 | 67.3 | 66.3 |
| 15 | 70.8 | 70.8 | 69.2 | 68.6 | 68.9 | 67.7 | 69.5 | 68.2 | 68.5 | 68.6 | 68.7 | 68.9 | 68.5 | 68.7 | --- | 94.3 | 71.1 | 70.1 | 68.0 | 64.9 | 67.0 | 66.5 | 66.5 | 66.8 | 66.8 | 67.9 | 67.7 | 67.7 | 68.4 | 68.4 | 68.1 | 68.4 | 68.2 | 68.5 | 67.0 |
| 16 | 69.7 | 69.7 | 68.9 | 68.1 | 68.9 | 67.6 | 69.2 | 68.1 | 68.3 | 68.3 | 68.6 | 68.6 | 68.4 | 68.4 | 94.3 | --- | 71.1 | 70.2 | 68.0 | 65.0 | 66.8 | 66.4 | 66.6 | 66.7 | 66.8 | 67.7 | 67.8 | 67.6 | 68.2 | 68.3 | 68.0 | 68.4 | 68.1 | 68.4 | 66.9 |
| 17 | 67.3 | 67.3 | 67.2 | 67.3 | 68.1 | 66.7 | 67.7 | 67.6 | 67.8 | 67.6 | 67.8 | 67.7 | 67.4 | 67.4 | 71.0 | 71.0 | --- | 71.0 | 70.3 | 66.3 | 68.6 | 68.5 | 65.9 | 66.0 | 65.4 | 66.0 | 65.4 | 66.1 | 66.1 | 66.2 | 66.0 | 66.2 | 65.7 | 66.0 | 65.8 |
| 18 | 67.1 | 67.1 | 67.4 | 67.2 | 68.5 | 66.9 | 67.7 | 67.4 | 68.4 | 67.9 | 68.1 | 67.9 | 67.6 | 67.6 | 70.0 | 70.1 | 71.1 | --- | 73.1 | 67.5 | 71.3 | 70.4 | 66.0 | 66.0 | 65.4 | 66.4 | 65.8 | 66.4 | 66.4 | 66.3 | 66.4 | 66.4 | 66.0 | 66.4 | 66.3 |
| 19 | 64.9 | 64.9 | 65.0 | 65.3 | 66.8 | 65.8 | 65.2 | 66.2 | 67.1 | 66.8 | 66.8 | 66.4 | 66.4 | 66.4 | 67.9 | 67.9 | 70.3 | 73.0 | --- | 68.8 | 71.9 | 71.6 | 65.3 | 65.3 | 64.5 | 64.9 | 64.1 | 64.9 | 64.6 | 64.8 | 64.7 | 64.6 | 64.4 | 64.8 | 65.0 |
| 20 | 64.5 | 64.5 | 64.7 | 65.1 | 66.9 | 66.9 | 64.7 | 65.7 | 66.1 | 66.1 | 65.9 | 65.8 | 65.8 | 65.9 | 65.1 | 64.9 | 66.8 | 67.6 | 68.9 | --- | 71.4 | 71.5 | 64.9 | 64.8 | 64.3 | 64.5 | 63.7 | 64.4 | 64.1 | 64.5 | 64.2 | 64.3 | 64.0 | 64.2 | 64.5 |
| 21 | 64.8 | 64.8 | 64.7 | 65.0 | 66.6 | 65.6 | 64.8 | 65.8 | 66.7 | 66.4 | 66.4 | 66.1 | 66.0 | 66.2 | 66.5 | 66.5 | 68.5 | 71.0 | 71.5 | 71.2 | --- | 89.0 | 64.8 | 64.7 | 64.0 | 64.4 | 63.9 | 64.7 | 64.4 | 64.5 | 64.5 | 64.5 | 64.0 | 64.4 | 64.5 |
| 22 | 64.4 | 64.4 | 64.4 | 64.7 | 66.2 | 65.4 | 64.6 | 65.5 | 66.6 | 66.2 | 66.0 | 65.7 | 65.8 | 66.0 | 66.3 | 66.0 | 68.4 | 70.3 | 71.5 | 71.3 | 89.1 | --- | 64.9 | 64.7 | 64.0 | 64.6 | 64.0 | 64.5 | 64.4 | 64.4 | 64.2 | 64.3 | 63.9 | 64.1 | 64.5 |
| 23 | 66.3 | 66.3 | 66.1 | 65.9 | 66.2 | 65.9 | 66.2 | 66.1 | 65.9 | 65.9 | 66.0 | 66.0 | 65.9 | 65.9 | 66.1 | 66.1 | 65.9 | 65.6 | 65.0 | 64.4 | 65.1 | 64.9 | --- | 85.1 | 73.7 | 74.3 | 73.4 | 74.2 | 70.5 | 70.9 | 70.3 | 70.7 | 70.1 | 70.6 | 69.2 |
| 24 | 66.6 | 66.6 | 66.7 | 66.4 | 66.7 | 66.2 | 66.8 | 66.7 | 66.3 | 66.4 | 66.5 | 66.5 | 66.4 | 66.3 | 66.5 | 66.5 | 66.7 | 66.2 | 65.5 | 65.0 | 65.7 | 65.6 | 85.4 | --- | 73.8 | 74.6 | 73.6 | 74.1 | 70.9 | 70.9 | 70.6 | 71.0 | 70.2 | 70.7 | 69.4 |
| 25 | 66.9 | 66.9 | 67.0 | 66.5 | 66.5 | 66.7 | 67.1 | 66.4 | 66.1 | 66.0 | 66.3 | 66.3 | 66.3 | 66.3 | 66.7 | 66.8 | 65.8 | 65.5 | 64.3 | 64.3 | 64.3 | 64.1 | 73.8 | 73.8 | --- | 75.8 | 75.3 | 75.6 | 71.7 | 71.5 | 71.3 | 72.2 | 71.2 | 71.7 | 69.3 |
| 26 | 67.6 | 67.6 | 67.8 | 67.1 | 67.3 | 67.2 | 67.6 | 66.9 | 67.0 | 67.0 | 67.1 | 67.3 | 67.1 | 67.1 | 67.5 | 67.4 | 66.8 | 66.4 | 65.3 | 64.4 | 65.1 | 64.9 | 74.3 | 74.5 | 75.7 | --- | 83.9 | 84.1 | 73.3 | 73.3 | 72.8 | 73.3 | 72.6 | 73.3 | 69.8 |
| 27 | 67.3 | 67.3 | 67.5 | 66.7 | 66.6 | 66.6 | 67.6 | 66.6 | 66.2 | 66.5 | 66.8 | 66.8 | 66.6 | 66.6 | 67.5 | 67.5 | 65.7 | 65.5 | 63.9 | 63.3 | 64.2 | 63.9 | 73.4 | 73.4 | 75.2 | 84.0 | --- | 80.5 | 72.9 | 72.9 | 72.7 | 73.3 | 72.7 | 73.1 | 69.4 |
| 28 | 67.3 | 67.3 | 67.4 | 66.9 | 67.2 | 67.0 | 67.4 | 67.1 | 66.9 | 66.9 | 67.1 | 67.1 | 67.0 | 67.1 | 67.5 | 67.4 | 66.4 | 66.2 | 65.0 | 64.6 | 65.1 | 65.0 | 74.2 | 74.1 | 75.3 | 84.1 | 80.5 | --- | 72.9 | 73.0 | 72.7 | 73.1 | 72.2 | 72.8 | 69.8 |
| 29 | 67.9 | 67.9 | 67.9 | 67.3 | 67.2 | 66.8 | 67.9 | 67.0 | 66.8 | 67.0 | 67.2 | 67.4 | 67.2 | 67.2 | 68.0 | 67.9 | 66.5 | 66.0 | 64.6 | 64.0 | 64.6 | 64.3 | 70.5 | 70.7 | 71.3 | 73.1 | 72.8 | 72.8 | --- | 91.7 | 88.4 | 86.0 | 80.9 | 82.0 | 69.2 |
| 30 | 68.3 | 68.3 | 68.2 | 67.5 | 67.6 | 66.8 | 68.0 | 67.3 | 67.1 | 67.2 | 67.5 | 67.4 | 67.4 | 67.3 | 67.9 | 68.0 | 66.7 | 66.4 | 65.0 | 64.4 | 65.0 | 64.5 | 70.7 | 70.7 | 71.3 | 73.1 | 73.0 | 72.9 | 92.0 | --- | 90.0 | 86.3 | 81.0 | 82.3 | 69.5 |
| 31 | 67.9 | 67.9 | 68.0 | 67.2 | 67.5 | 66.8 | 67.6 | 67.1 | 66.9 | 67.1 | 67.1 | 67.4 | 67.2 | 67.2 | 67.9 | 67.8 | 66.4 | 66.3 | 64.7 | 64.4 | 65.0 | 64.4 | 70.4 | 70.5 | 71.4 | 73.0 | 72.6 | 72.6 | 88.4 | 89.8 | --- | 85.4 | 80.3 | 81.9 | 69.0 |
| 32 | 68.0 | 68.0 | 68.0 | 67.4 | 67.4 | 66.8 | 68.0 | 67.3 | 67.1 | 67.1 | 67.4 | 67.3 | 67.3 | 67.3 | 67.9 | 68.0 | 66.4 | 66.1 | 64.4 | 64.0 | 64.5 | 64.2 | 70.7 | 71.2 | 72.5 | 73.3 | 73.2 | 72.9 | 86.5 | 86.4 | 85.5 | --- | 81.1 | 82.5 | 69.4 |
| 33 | 68.0 | 68.0 | 68.2 | 67.1 | 67.1 | 67.0 | 67.9 | 66.9 | 66.7 | 66.8 | 67.1 | 67.1 | 67.0 | 66.9 | 67.9 | 67.7 | 66.4 | 65.9 | 64.3 | 64.3 | 64.6 | 64.2 | 70.0 | 70.0 | 71.0 | 72.6 | 72.7 | 72.2 | 81.0 | 81.0 | 80.2 | 81.1 | --- | 82.9 | 68.8 |
| 34 | 68.2 | 68.2 | 68.4 | 67.5 | 67.4 | 67.1 | 68.2 | 67.3 | 66.9 | 67.4 | 67.5 | 67.6 | 67.2 | 67.2 | 68.2 | 68.1 | 66.6 | 66.3 | 64.9 | 64.4 | 65.0 | 64.5 | 70.7 | 70.6 | 71.5 | 73.2 | 73.0 | 72.9 | 82.2 | 82.3 | 81.8 | 82.4 | 82.9 | --- | 69.5 |
| 35 | 66.7 | 66.7 | 66.6 | 66.4 | 66.5 | 65.9 | 66.6 | 66.3 | 66.2 | 66.5 | 66.5 | 66.4 | 66.3 | 66.3 | 66.8 | 66.7 | 66.3 | 65.9 | 65.0 | 64.5 | 65.0 | 64.8 | 69.2 | 69.1 | 69.4 | 69.8 | 69.4 | 69.8 | 69.0 | 69.3 | 69.0 | 69.4 | 68.9 | 69.4 | --- |

Additional phenotypic testing was performed as described previously [20–24]. Nitrate reduction, indoxyl acetate hydrolysis, urea hydrolysis, hippurate hydrolysis, $H_2S$ production on TSI agar, and oxidase, catalase, alkaline phosphatase, and $\gamma$-glutamyl transferase activity were determined. In addition to this, growth in the presence of 1 % glycine, $\alpha$-haemolysis on sheep blood agar, ability to grow in absence of $H_2$ (86.9 % $N_2$, 7.1 % $CO_2$, and 6 % $O_2$), and resistance to nalidixic acid (30 µg) and cephalothin (30 µg) were evaluated, and the isolates were cultured at various temperatures, atmospheres and on several agar media, including Blaser–Wang, CCD, MacConkey, Preston, and Skirrow agar. Motility was assessed by phase-contrast microscopy. The isolates displayed phenotypic characteristics distinct from all other *Campylobacter* taxa (Table 3). Based on the lack of motility, growth at 25 °C and growth on MacConkey agar, the isolates could be distinguished from all other *Campylobacter* taxa. The lack of motility was supported by the absence of all flagellar genes in the genomes.

Similar to the pinniped-associated *C. pinnipediorum*, a urease-encoding locus was present in the genomes of the isolates, which supports survival in an acidic environment. A BLAST search identified *Campylobacter* 16S rRNA gene sequences which shared 98–99 % sequence homology with the 16S rRNA sequences of the isolates described in this study in oral, gastric and rectal samples from California sea lions (*Zalophus californianus*), indicating the presence of a highly related *Campylobacter* lineage in a gastric niche, and in a wider geographical and host range [4]. The observed high loads of this taxon in the intestinal microbiome of common seals in the absence of obvious adverse health effects and the absence of multiple known virulence factors associated with pathogenic *Helicobacter* and *Campylobacter* species suggest a commensal relationship with its host.

In conclusion, the results from this polyphasic taxonomic study clearly demonstrate that the isolates recovered from common seals comprise a novel species distinct from all other currently known *Campylobacter* species, based on 16S rRNA gene and whole genome-based phylogeny and phenotypic characteristics. The name *Campylobacter blaseri* sp. nov. is proposed for these isolates.

## DESCRIPTION OF *CAMPYLOBACTER BLASERI* SP. NOV.

*Campylobacter blaseri* sp. nov. (bla′se.ri. N.L. gen. n. *blaseri*, named in honour of Dr Martin J. Blaser, a microbiologist who has studied the *Campylobacter* and *Helicobacter* species for 40 years).

**Table 3.** Characteristics differentiating *C. blaseri* sp. nov. from other *Campylobacter* taxa

Taxa: 1, *Campylobacter blaseri* sp. nov. (*n*=10); 2, *C. avium*; 3, *C. canadensis*; 4, *C. coli*; 5, *C. concisus*; 6, *C. corcagiensis*; 7, *C. cuniculorum*; 8, *C. curvus*; 9, *C. fetus* subsp. *fetus*; 10, *C. fetus* subsp. *testudinum;* 11, *C. fetus* subsp. *venerealis*; 12, *C. geochelonis*; 13, *C. gracilis*; 14, *C. helveticus*; 15, *C. hepaticus*; 16, *C. hominis*; 17, *C. hyointestinalis* subsp. *hyointestinalis*; 18, *C. hyointestinalis* subsp. *lawsonii*; 19, *C. iguaniorum*; 20, *C. insulaenigrae*; 21, *C. jejuni* subsp. *doylei*; 22, *C. jejuni* subsp. *jejuni*; 23, *C. lanienae*; 24, *C. lari* subsp. *concheus*; 25, *C. lari* subsp. *lari*; 26, *C. mucosalis*; 27, *C. ornithocola*; 28, *C. peloridis*; 29, *C. pinnipediorum* subsp. *caledonicus*; 30, *C. pinnipediorum* subsp. *pinnipediorum*; 31, *C. rectus*; 32, *C. showae*; 33, *C. sputorum*; 34, *C. subantarcticus*; 35, *C. upsaliensis*; 36, *C. ureolyticus*; 37, *C. volucris*. Characteristics of reference taxa were adapted from previous species descriptions [3, 26–33]. +, 90–100 %; (+), 75–89 %; V, 26–74 %; (−), 11–25 %; −, 0–10 %; ND, not determined.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Motility | - | + | + | + | + | - | + | + | + | + | + | + | - | + | + | - | + | + | + | + | + | + | + | + | + | + | ND | ND | + | + | + | + | + | ND | + | - | ND |
| Oxidase | + | + | + | + | V | + | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | V | + | + | + | + | + |
| Catalase | + | + | V | + | - | + | + | - | + | + | (+) | + | (-) | - | + | - | + | + | + | + | V | + | + | + | + | - | + | + | - | + | (-) | + | V† | + | - | V | + |
| Alkaline phosphatase | + | - | - | - | V | + | - | V | - | ND | - | - | - | - | ND | - | - | (-) | - | ND | - | - | + | - | - | (+) | - | ND | + | + | - | - | - | ND | - | - | - |
| Urease | + | - | V | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | ND | + | + | - | - | V† | ND | - | + | ND |
| Nitrate reduction | + | + | V | + | (-) | (+) | + | + | + | + | (+) | + | (+) | + | V | - | + | + | + | + | - | + | + | + | + | (-) | V | ND | + | + | + | + | (+) | + | + | + | + |
| Hippurate hydrolysis | - | + | - | - | - | - | - | (-) | - | - | - | + | - | - | V | - | - | - | - | - | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Indoxyl acetate hydrolysis | + | + | - | + | - | V | + | V | - | - | - | - | (+) | + | + | - | - | - | - | - | + | + | - | ND | - | - | - | ND | - | - | + | V | - | - | + | V | - |
| γ-Glutamyl transferase | - | - | + | - | - | ND | - | ND | - | ND | - | ND | ND | - | ND | ND | - | - | + | ND | - | - | ND | ND | - | ND | ND | ND | + | + | ND | ND | - | ND | - | ND | ND |
| $H_2S$ production (TSI) | + | - | V | - | - | + | - | (-) | - | - | - | - | - | - | - | - | + | + | + | - | - | - | - | ND | - | + | - | ND | + | + | - | V | + | - | - | - | - |
| α-Haemolysis | - | - | - | (-) | (-) | - | + | (-) | - | - | V | - | - | + | - | - | V | V | + | ND | + | + | + | ND | + | - | - | ND | + | + | + | + | + | + | + | V | ND |
| Growth at/in/on: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 18-22°C (microaerobic) | - | ND | ND | - | - | ND | ND | - | (+) | + | (-) | - | - | - | ND | ND | (-) | - | + | ND | - | - | ND | ND | - | - | ND | ND | - | - | - | - | - | - | - | ND | - |
| 25°C (microaerobic) | + | - | - | - | - | ND | - | - | + | + | + | + | - | - | - | - | (-) | - | + | - | - | - | - | - | - | - | - | - | + | + | - | - | - | - | - | - | - |
| 37°C (microaerobic) | + | + | + | + | + | + | + | V | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | V | + | + | + | + | + |
| 42°C (microaerobic) | + | + | + | + | (+) | + | (+) | V | (+) | V | - | - | V | + | + | (-) | + | + | - | - | - | + | + | + | + | + | + | + | - | - | (-) | V | + | + | + | V | + |
| 37°C (anaerobic) | + | - | + | - | + | + | - | + | (-) | + | V | + | + | - | - | + | - | + | + | - | - | - | + | ND | - | + | + | ND | + | + | + | + | + | + | - | + | + |
| 37°C (aerobic) | - | - | - | - | - | - | - | - | - | V | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| CCDA | - | - | + | + | (-) | ND | (+) | (+) | + | + | + | + | V | + | ND | ND | + | + | + | ND | + | + | ND | + | + | + | ND | + | - | - | - | + | (+) | ND | + | ND | ND |
| MacConkey agar | + | - | + | V | - | - | - | (+) | (+) | (+) | (+) | - | (+) | - | - | - | V | V | ND | ND | - | - | + | ND | ND | (+) | ND | ND | ND | ND | - | + | V | (-) | - | V | +* |
| Glycine (1%) | +* | - | V | (+) | (-) | + | - | + | + | + | (-) | + | + | V | + | + | + | V | + | + | (-) | + | - | (+) | + | (-) | + | + | - | V | + | V | + | (+) | + | + | - |
| Resistance to: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Nalidixic acid (30 μg) | - | - | V | - | (+) | + | V | + | + | + | V | + | V | - | + | V | + | + | + | + | - | - | + | - | (+) | (+) | ND | (+) | - | - | (+) | - | (+) | + | - | - | + |
| Cephalothin (30 μg) | - | + | - | + | - | - | (+) | - | - | ND | - | - | - | - | (+) | - | (-) | - | - | + | - | + | + | + | + | - | ND | (-) | - | - | - | - | - | - | (-) | - | + |
| $H_2$ requirement | - | V | - | - | + | - | - | + | - | ND | - | - | + | - | - | + | V | V | - | ND | - | - | - | ND | - | + | - | ND | - | - | + | + | - | ND | - | + | ND |
| DNA G+C content (mol%) | 29 | 35 | ND | 31 | 37 - 41 | 32 | 32 | 45 - 46 | 33 - 35 | 33 | 33 - 34 | 34 | 44 - 46 | 34 | 28 | 32 - 33 | 35 - 36 | 31 - 33 | 36 | ND | 31 | 30 - 31 | 36 | 30 | 29 - 30 | 36 - 38 | 30 | 29 | 31 | 30 | 45 - 46 | 44 - 46 | 29 - 33 | 30 | 32 - 36 | 28 - 30 | 29 |

*Weak growth.

†Test results differ between *C. sputorum* biovars *sputorum* (catalase- and urease-negative), *paraureolyticus* (catalase-negative, urease-positive), and *fecalis* (catalase-positive, urease-negative).

Cells are Gram-reaction-negative straight to slightly curved non-motile rods. After incubation on Columbia agar with 5 % sheep blood in a microaerobic atmosphere at 37 °C for 72 h, colonies of most isolates (9/10) appear transparent to greyish white and are 1–2 mm, raised and circular with entire margins and a smooth surface. Morphology is similar under anaerobic conditions, but colonies appear transparent and are smaller (<1 mm). One isolate displays raised and circular colonies with undulate margins and an irregular surface. Under anaerobic conditions, this isolate displays flat transparent colonies with a slightly elevated centre. None of the isolates display α-haemolysis. In a microaerobic atmosphere, growth is observed at 25, 37 and 42 °C, but not at room temperature (18–22 °C). Compared to microaerobic conditions, growth is slightly inhibited under anaerobic conditions, but no growth is detected under aerobic conditions (ambient air) at 37 °C. Growth is slightly inhibited in the absence of $H_2$ at microaerobic conditions. In a microaerobic atmosphere at 37 °C, growth is normal on Skirrow agar, slightly inhibited on Preston and MacConkey agar, and none detected on CCD and Blaser–Wang agar. Weak growth in the presence of 1 % glycine. All isolates are positive for urea hydrolysis, oxidase, catalase and alkaline phosphatase activity, nitrate reduction, and for production of $H_2S$ on TSI agar, but are negative for hydrolysis of hippurate and γ-glutamyl transferase activity. All isolates are susceptible to cephalothin, and most (9/10) are susceptible to nalidixic acid. Pathogenicity is unknown, but it presumably represents a commensal in pinnipeds.

The species type strain is 17S00004-5$^T$ (=LMG 30333$^T$ =CCUG 71276$^T$), which was isolated from a common seal (*Phoca vitulina*) in 2017.

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## Conflicts of interest

The authors declare that there are no conflicts of interest.

## Ethical statement

The procedures conducted on the animals in the study were combined with routine veterinary diagnostic and therapeutic acts and were not considered to cause any additional discomfort. Consequently, the study was not considered an animal experiment under the Dutch 'Experiments on Animals Act', making assessment by an animal ethics committee unnecessary.

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