

# *Campylobacter pinnipediorum* sp. nov., isolated from pinnipeds, comprising *Campylobacter pinnipediorum* subsp. *pinnipediorum* subsp. nov. and *Campylobacter pinnipediorum* subsp. *caledonicus* subsp. nov.

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## Abstract

During independent diagnostic screenings of otariid seals in California (USA) and phocid seals in Scotland (UK), *Campylobacter*-like isolates, which differed from the established taxa of the genus *Campylobacter*, were cultured from abscesses and internal organs of different seal species. A polyphasic study was undertaken to determine the taxonomic position of these six isolates. The isolates were characterized by 16S rRNA gene and AtpA sequence analysis and by conventional phenotypic testing. The whole-genome sequences were determined for all isolates, and the average nucleotide identity (ANI) was determined. The isolates formed a separate phylogenetic clade, divergent from all other taxa of the genus *Campylobacter* and most closely related to *Campylobacter mucosalis*. Although all isolates showed 100% 16S rRNA gene sequence homology, AtpA and ANI analyses indicated divergence between the otariid isolates from California and the phocid isolates from Scotland, which warrants subspecies status for each clade. The two subspecies could also be distinguished phenotypically on the basis of catalase activity. This study shows clearly that the isolates obtained from pinnipeds represent a novel species within the genus *Campylobacter*, for which the name *Campylobacter pinnipediorum* sp. nov. is proposed. Within this novel species, the Californian isolates represent a separate subspecies, for which the name *C. pinnipediorum* subsp. *pinnipediorum* subsp. nov. is proposed. The type strain for both this novel species and subspecies is RM17260<sup>T</sup> (=LMG 29472<sup>T</sup>=CCUG 69570<sup>T</sup>). The Scottish isolates represent another subspecies, for which the name *C. pinnipediorum* subsp. *caledonicus* subsp. nov. is proposed. The type strain of this subspecies is M302/10/6<sup>T</sup> (=LMG 29473<sup>T</sup>=CCUG 68650<sup>T</sup>).

Of all currently recognized species of the genus *Campylobacter*, at least five are predominantly associated with animals found in marine environments. *Campylobacter lari*, *C. peloridis*, *C. subantarcticus* and *C. volucris* [1–3] have all been isolated from shellfish and/or marine birds, whereas *C. insulaenigrae* has been isolated from marine mammals [4]. These predominantly thermotolerant species are all closely related and belong to the same clade, which includes the human pathogen *Campylobacter jejuni*. In contrast, species of the genus *Campylobacter* from the clade to which *Campylobacter concisus* and *Campylobacter mucosalis* belong are not or are rarely associated with marine animals; many have been isolated from the oral cavity of terrestrial vertebrates,

including humans [5]. We describe a novel, urease-positive *Campylobacter* species, related to *C. mucosalis* and *C. concisus*, which has been isolated from pinnipeds. Furthermore, this species is proposed to contain two subspecies.

Pinnipeds comprise the families Odobenidae (walruses), Otariidae (eared seals) and Phocidae (earless seals). Six *Campylobacter*-like isolates not belonging to any of the established taxa of the genus *Campylobacter* were obtained from pinnipeds during independent diagnostic screenings in California (USA) and Scotland (UK). Three isolates were obtained from internal organs and an abscess of California sea lion (*Zalophus californianus*) juveniles from a seal

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**Keywords:** *Campylobacter*; novel species; pinnipeds; whole genome sequencing; average nucleotide identity; urease.

**Abbreviations:** ANI, average nucleotide identity; DDH, DNA-DNA hybridization.

The GenBank/EMBL/DBJ accession numbers for the whole-genome sequences of strains RM17260<sup>T</sup>, RM17261, RM17262, M203/00/3, M302/10/6<sup>T</sup> and M341/11/05 are CP012546, CP012547, CP012548, MBGA00000000, CP017018 and CP017258, respectively.

rehabilitation centre in San Diego. Three isolates were obtained from abscesses of stranded deceased common seal (*Phoca vitulina*) and grey seal (*Halichoerus grypus*) juveniles from the coastal regions of Scotland. Noteworthy, from two of these abscesses no organisms other than the *Campylobacter*-like organisms were isolated.

Initial characterization using amplified fragment length polymorphism [6] and 16S rRNA gene sequencing [7] indicated that these isolates were different from all other species of the genus *Campylobacter*, but most closely related to *C. mucosalis*. A recent study analysing the microbiota of sea mammals [8] identified *Campylobacter* 16S rRNA gene sequences in both oral and gastric samples from California sea lions, but not from common bottlenose dolphins (*Tursiops truncatus*). These 16S rRNA gene sequences shared 99–100 % sequence homology with the 16S rRNA gene sequences of the isolates described in this study.

A polyphasic study was undertaken to determine the taxonomic position of these six isolates. Whole-genome sequencing was performed on all isolates, and the average nucleotide identity (ANI) was determined. Comparisons based on 16S rRNA gene and AtpA protein sequences were made to determine the taxonomic position of the isolates. Phenotypic characteristics were determined by conventional biochemical testing for all six isolates.

Apart from the six isolates used for extended taxonomic analysis, five additional isolates belonging to the genus *Campylobacter* with identical 16S rRNA gene sequences were obtained from the oral and rectal cavities of California sea lions and from an abscess of a Steller sea lion (*Eumetopias jubatus*). In support of the extended taxonomic analysis, these isolates were used for ANI and evaluated in the discriminating phenotypic tests. Characteristics of all strains are summarized in Table 1.

Complete genome sequences for strains RM17260<sup>T</sup>, RM17261, RM17262 and M302/10/6<sup>T</sup> were obtained as described by Miller *et al.* (unpublished). Briefly, initial sequencing was performed on a Roche 454 GS-FLX+ Genome Sequencer (Roche Life Science). The 454 sequencing reads were assembled into single scaffolds using the Roche Newbler assembler (version 2.6), and base calls were validated using Illumina MiSeq (Illumina) reads. Additional sequencing was performed for the above four strains and *de novo* for strains M341/11/05 and M203/00/3 using a PacBio RS sequencer (Pacific Biosciences) to generate complete, closed genomes. Draft genomes for strains RM18812, RM18813, RM18906, 1105248A and 03036546 were obtained using the Illumina MiSeq. Sequencing reads were assembled using Newbler or SPAdes (version 3.1.1). The genome sequences have been deposited in GenBank; accession numbers are listed in Table 1.

The taxonomic position of all strains was determined by 16S rRNA gene sequence comparison. The 16S rRNA gene sequences ( $\geq 1339$  bp) were extracted from the whole-genome sequences of the strains or obtained from EzTaxon [9] for the other species of the genus *Campylobacter*. Sequence alignment and dendrogram reconstruction were performed using CLUSTAL X (version 2.1) and MEGA version 6.05 [10]. A neighbour-joining dendrogram containing all taxa of the genus *Campylobacter* was reconstructed (Fig. 1). Bootstrap values were determined using 500 repetitions. The 16S rRNA gene sequence from *Arcobacter butzleri* strain RM4018 was used to root the tree. The 16S rRNA gene sequence similarity between the pinniped-associated strains was 100 %, while the sequence similarity between these strains and the most closely related species, *C. concisus* and *C. mucosalis*, was 96–97 %.

For improved taxonomic resolution [11], full AtpA protein sequences were extracted from the whole-genome sequences

**Table 1.** Features of the *Campylobacter pinnipediorum* sp. nov. strains used in this study

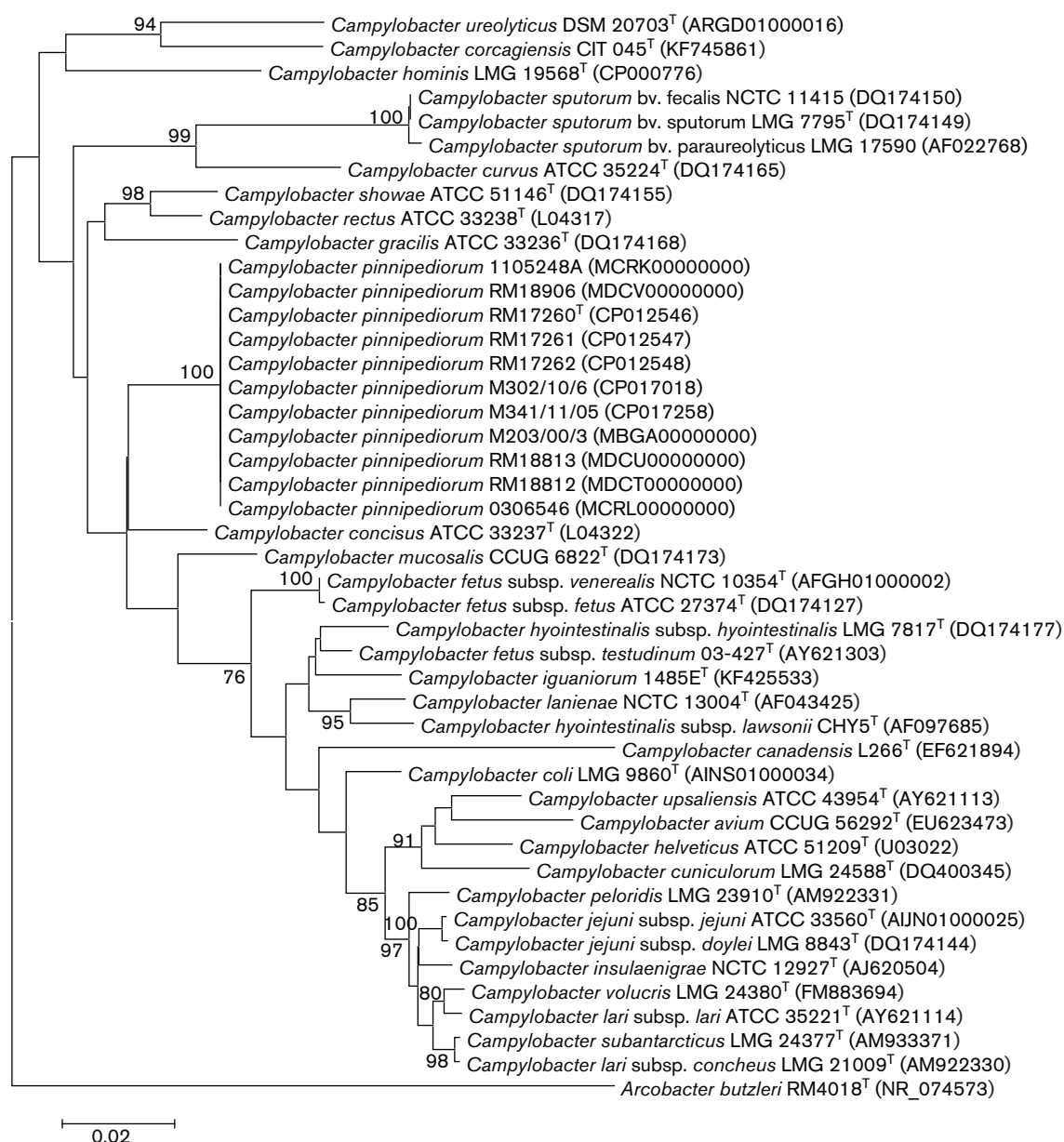
Subspecies	Strain	Alternative designation	Isolation date	Location	Host species	Source	Sex	Age	Accession no.
<i>C. pinnipediorum</i> subsp. <i>pinnipediorum</i>	RM17260 <sup>T</sup>	LMG 29472 <sup>T</sup> CCUG 69570 <sup>T</sup> SW130133 <sup>T</sup>	7-2-2013	San Diego, California, USA	California sea lion ( <i>Z. californianus</i> )	Abscess	NA	Juvenile	CP012546
<i>C. pinnipediorum</i> subsp. <i>pinnipediorum</i>	RM17261	SW130167	NA	San Diego, CA, USA	California sea lion ( <i>Z. californianus</i> )	Lung	NA	Juvenile	CP012547
<i>C. pinnipediorum</i> subsp. <i>pinnipediorum</i>	RM17262	SW130202	NA	San Diego, CA, USA	California sea lion ( <i>Z. californianus</i> )	Abscess fluid	NA	Juvenile	CP012548
<i>C. pinnipediorum</i> subsp. <i>pinnipediorum</i>	RM18812	SWCZC1617B	1-25-2016	San Diego, CA, USA	California sea lion ( <i>Z. californianus</i> )	Oral	F	Juvenile	MDCT00000000
<i>C. pinnipediorum</i> subsp. <i>pinnipediorum</i>	RM18813	SWCZC1626B	1-29-2016	San Diego, CA, USA	California sea lion ( <i>Z. californianus</i> )	Rectal	F	Juvenile	MDCU00000000
<i>C. pinnipediorum</i> subsp. <i>pinnipediorum</i>	RM18906	SWCZC1639B	2-27-2016	San Diego, CA, USA	California sea lion ( <i>Z. californianus</i> )	Oral	M	Juvenile	MDCV00000000
<i>C. pinnipediorum</i> subsp. <i>pinnipediorum</i>	1105248A	16S02911-1	5-2011	Laguna Beach, CA, USA	California sea lion ( <i>Z. californianus</i> )	Abscess	NA	NA	MCRK00000000
<i>C. pinnipediorum</i> subsp. <i>pinnipediorum</i>	0306546	16S02912-1	3-9-2003	Resurrection Bay, AK, USA	Steller sea lion ( <i>E. jubatus</i> )	Abscess	F	Juvenile	MCRL00000000
<i>C. pinnipediorum</i> subsp. <i>caledonicus</i>	M203/00/3	NA	3-11-2000	Inverness, Scotland, UK	Common seal	Shoulder abscess	M	Juvenile	MBGA00000000
<i>C. pinnipediorum</i> subsp. <i>caledonicus</i>	M302/10/6 <sup>T</sup>	LMG 29473 <sup>T</sup> CCUG 68650 <sup>T</sup>	11-15-2010	Inverness, Scotland, UK	Grey seal ( <i>H. grypus</i> )	Lung abscess	F	Juvenile	CP017018
<i>C. pinnipediorum</i> subsp. <i>caledonicus</i>	M341/11/05	NA	11-29-2011	Inverness, Scotland, UK	Common seal ( <i>P. vitulina</i> )	Submaxillary abscess	F	Juvenile	CP017258

NA, Data not available.

or obtained from GenBank. Alignment and dendrogram reconstruction were performed as described above; the AtpA sequence from *A. butzleri* strain RM4018 was used to root the tree. Consistent with the 16S rRNA gene sequence comparison, the pinniped-associated strains formed a clade distinct from other taxa of the genus *Campylobacter* (Fig. 2). Furthermore, a clear distinction could be observed between the strains isolated in California and Scotland.

As an alternative to DNA–DNA hybridization (DDH), the ANI has been suggested [12, 13]. A DDH species delineation

of 70 % corresponds to about 95 % ANI [14]. Using JSpecies v. 1.2.1 [15], pairwise ANI values based on whole-genome sequences were calculated for all the unidentified strains belonging to the genus *Campylobacter* and the most closely related species: *C. concisus* (strain 13826; accession no. CP000792), *C. curvus* (strain 525.92; accession no. CP000767), *C. mucosalis* (strain DSM 21682<sup>T</sup>; accession no. JHQQ01), *C. rectus* (strain ATCC 33238<sup>T</sup>; accession no. ACFU00000000) and *C. showae* (strain ATCC 51146<sup>T</sup>; accession no. ACVQ00000000). While ANI for digital DDH assessments of members of the genus *Campylobacter* and related

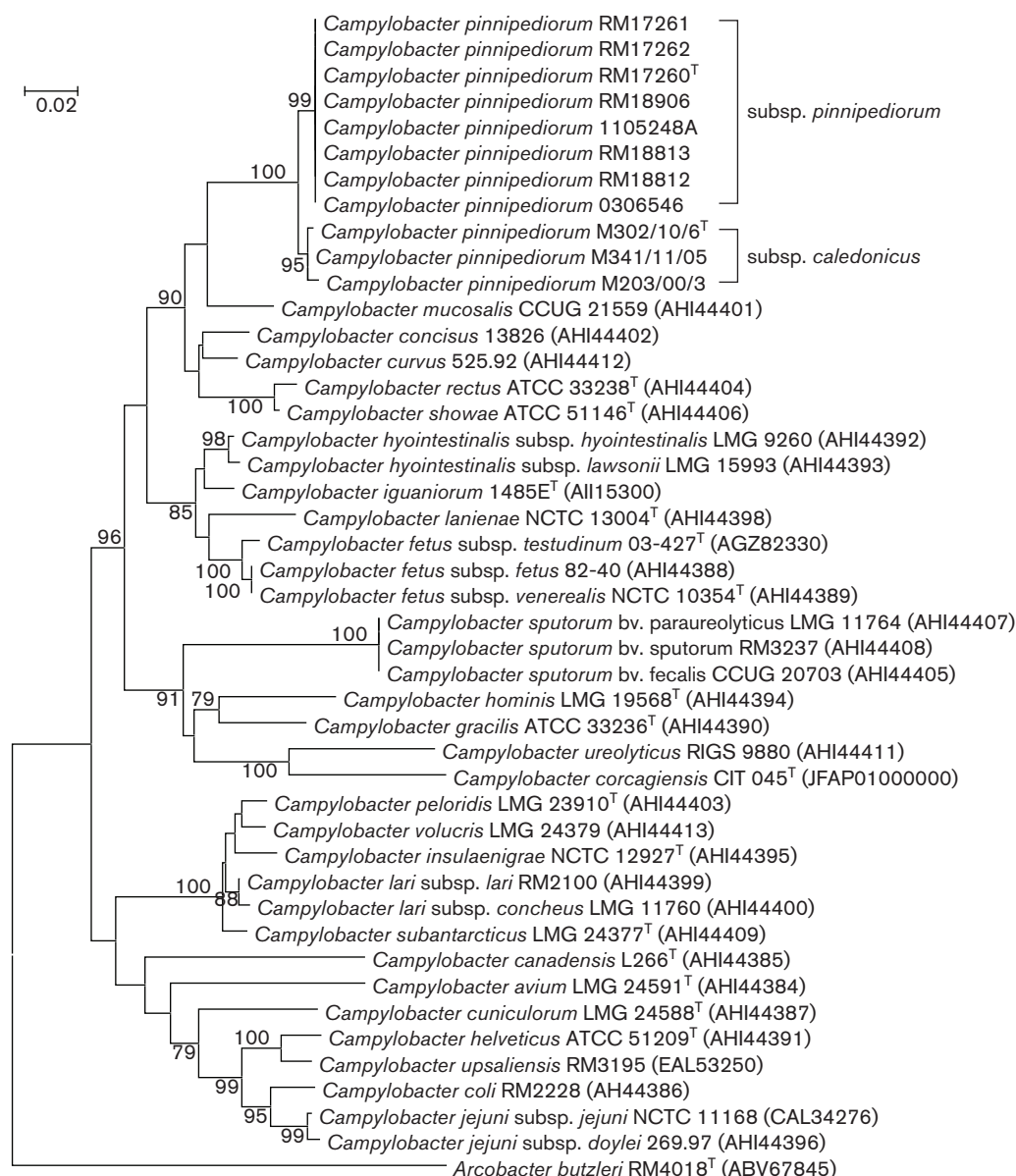


**Fig. 1.** Neighbour-joining phylogenetic dendrogram based on 16S rRNA gene sequences. Bootstrap values (≥75 %) based on 500 replications are indicated at the nodes. Bar, 0.02 substitutions per nucleotide position.

species has not been tested, the ANI between the pinniped-associated strains and the most closely related species (*C. mucosalis*) was maximally 71 %, which is well below the 95 % species cut-off suggested by Goris and colleagues [14] and similar to ANI values observed between the pinniped-associated strains and the other related species (Table 2). Strains originating from either California or Scotland were highly homologous amongst each other (ANI  $\geq 98$  %). However, 94–95 % ANI was observed between the Californian and Scottish strains, indicating divergence between both groups of strains on a genomic level. The taxonomic position of the novel

strains belonging to the genus *Campylobacter* was further supported by a core genome phylogeny which included these strains and related taxa of the genus *Campylobacter* (Miller et al., unpublished).

The genetic analyses presented here indicate that the pinniped-associated strains form a distinct clade which is clearly separated from the closest known relatives. Based on the 100 % 16S rRNA gene sequence similarity, all strains examined clearly belong to the same species. However, the divergence observed between the Californian and Scottish strains, based on the ANI, AtpA and core genome phylogeny,



**Fig. 2.** Neighbour-joining phylogenetic dendrogram based on AtpA protein sequences. Bootstrap values ( $\geq 75$  %) based on 500 replications are indicated at the nodes. *Campylobacter pinnipediorum* sp. nov. genome sequence accession numbers are as listed in Fig. 1. Bar, 0.02 substitutions per amino acid position.

warrants subspecies status for each group. The 94–95 % ANI observed between the Californian and Scottish strains is consistent with the subspecies divergence observed in other species of the genus *Campylobacter*, such as *Campylobacter fetus* (8 % divergence between *C. fetus* subsp. *fetus* and *C. fetus* subsp. *testudinum*) and *Campylobacter hyointestinalis* (6 % divergence between *C. hyointestinalis* subsp. *hyointestinalis* and *C. hyointestinalis* subsp. *lawsonii*) [16].

The DNA G+C content was determined on the basis of whole-genome sequences using Artemis v.13.2 (Wellcome Trust Sanger Institute, UK; [17]). All strains had a DNA G+C content varying between 30.4 and 31.0 mol%, which is within the range observed within the genus *Campylobacter* (Table 3).

Additional phenotypic testing was performed as described previously [18–21]. Oxidase activity, catalase activity, nitrate reduction, indoxyl acetate hydrolysis, urea hydrolysis, hippurate hydrolysis and H<sub>2</sub>S production on TSI agar were determined. In addition to this, growth with 1 % glycine,  $\alpha$ -haemolysis on sheep blood agar, H<sub>2</sub> requirement and resistance to nalidixic acid (30  $\mu$ g) and cefalotin (30  $\mu$ g) were evaluated. Strains were grown at various temperatures and atmospheres and on different agar media. The novel strains displayed phenotypic characters distinct from all other taxa of the genus *Campylobacter*. All strains displayed urease activity, which may be related to a gastric niche. Indeed, 99–100 % 16S rRNA gene sequence similarity was observed between the strains and uncultured bacteria from the gastric microbiota of California sea lions [8]. Differentiating characteristics for the strains tested and other taxa of the genus

*Campylobacter* are summarized in Table 3. Based on urease activity, H<sub>2</sub>S production on TSI agar, nitrate reduction, growth at 25 °C in a microaerobic atmosphere and  $\alpha$ -haemolysis, the strains can be distinguished from all other described taxa of the genus *Campylobacter*. Results of the discriminatory phenotypic tests were identical for the five additional *Campylobacter* strains. Catalase activity was observed in all strains originating from otariid seals in California, but not in strains originating from phocid seals in Scotland, supporting the existence of two subspecies. Urease-positive *Campylobacter lari* (UPTC) is not, as yet, a defined taxon within the genus *Campylobacter* [1, 22–24]. Nevertheless, since such strains are also urease positive they could potentially share the same phenotypic profile as the pinniped-associated strains. Therefore, their phenotypic characteristics were also analysed, based on *C. lari* strains NCTC 11845, CCUG 22395, RM16701 and RM16712 [25]. UPTC strains could not grow at 25 °C microaerobically and did not produce H<sub>2</sub>S on TSI agar; thus, the pinniped-associated strains could also be readily distinguished from urease-positive *C. lari*.

In conclusion, the results from this polyphasic taxonomic study clearly demonstrate that the isolates recovered from pinnipeds comprise a novel species distinct from all other currently known species of the genus *Campylobacter*, based on 16S rRNA gene, AtpA and whole-genome sequence comparisons and biochemical properties. The name *Campylobacter pinnipediorum* sp. nov. is proposed for these strains. Within this novel species, strains originating from otariid seals in California form a separate subspecies, for which the name *C. pinnipediorum* subsp. *pinnipediorum*

**Table 2.** ANI values (percentages) based on BLAST for *Campylobacter pinnipediorum* sp. nov. and the most closely related species of the genus *Campylobacter*

Strains: 1, RM17260<sup>T</sup>; 2, RM17261; 3, RM17262; 4, RM18812; 5, RM18813; 6, RM18906; 7, 1105248A; 8, 0306546; 9, M302/10/6<sup>T</sup>; 10, M341/11/05; 11, M203/00/3; 12, *C. concisus* 13826; 13, *C. curvus* 525.92; 14, *C. mucosalis* ATCC 49352; 15, *C. rectus* ATCC 33238<sup>T</sup>; 16, *C. showae* ATCC 51146<sup>T</sup>. Strains 1–8, *C. pinnipediorum* subsp. *pinnipediorum* subsp. nov.; strains 9–11, *C. pinnipediorum* subsp. *caledonicus* subsp. nov.

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	100	98	98	98	98	97	98	98	94	94	94	70	68	71	67	67
2	98	100	98	98	98	97	98	98	94	94	94	70	68	71	66	67
3	98	98	100	98	98	97	98	98	95	94	94	70	68	71	66	67
4	98	98	98	100	98	97	98	98	94	94	94	69	68	71	66	67
5	98	98	98	98	100	97	98	98	94	94	94	69	68	71	66	67
6	97	97	97	97	97	100	97	97	95	95	95	69	68	71	66	67
7	98	98	98	98	98	97	100	98	94	94	94	69	68	71	66	66
8	98	98	98	98	98	97	98	100	94	94	94	69	68	71	66	67
9	94	94	94	94	94	95	94	94	100	99	99	69	68	71	66	67
10	94	94	94	94	94	95	94	94	99	100	99	69	68	71	66	67
11	94	94	94	94	94	95	94	94	99	99	100	69	68	71	66	67
12	69	69	69	70	69	70	69	69	69	69	69	100	74	71	71	71
13	68	68	68	68	68	68	68	68	68	68	68	74	100	70	72	72
14	71	71	71	71	71	71	71	71	71	71	71	71	70	100	68	69
15	66	66	66	66	66	66	66	66	66	66	66	71	72	69	100	89
16	67	67	67	67	67	67	67	67	67	67	67	71	72	69	89	100

**Table 3.** Characteristics differentiating *Campylobacter pinnipediorum* sp. nov. from other taxa of the genus *Campylobacter*

Taxa: 1, *C. pinnipediorum* subsp. nov. (n=3); 2, *C. pinnipediorum* subsp. *caledonicus* subsp. nov. (n=3); 3, *C. avium*; 4, *C. canadensis*; 5, *C. coli*; 6, *C. concisus*; 7, *C. coraciensis*; 8, *C. cuniculorum*; 9, *C. curvus*; 10, *C. fetus* subsp. *fetus*; 11, *C. fetus* subsp. *testudinum*; 12, *C. fetus* subsp. *venerealis*; 13, *C. gracilis*; 14, *C. helveticus*; 15, *C. hominis*; 16, *C. hyointestinalis* subsp. *hyointestinalis*; 17, *C. hyointestinalis* subsp. *lawsonii*; 18, *C. iguaniorum*; 19, *C. insulaenigræ*; 20, *C. jejuni* subsp. *doylei*; 21, *C. jejuni* subsp. *jejuni*; 22, *C. lari* subsp. *concheus*; 23, *C. lari* subsp. *lari*; 24, *C. lari* subsp. *lari*; 25, *C. mucosalis*; 26, *C. peloridis*; 27, *C. rectus*; 28, *C. showae*; 29, *C. sputorum*; 30, *C. subantarcticus*; 31, *C. upsaliensis*; 32, *C. ureolyticus*; 33, *C. volucris*. Characteristics of reference taxa were adapted from previous species descriptions [26–28]. +, 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
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urease	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hippurate hydrolysis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Indoxyl acetate hydrolysis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
γ-Glutamyl transferase	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H <sub>2</sub> S production (TSI)	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
α-Haemolysis	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at/in/on:																																	
18–22 °C (microaerobic)	–	–	ND	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
25 °C (microaerobic)	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
37 °C (microaerobic)	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
42 °C (microaerobic)	–	–	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
37 °C (anaerobic)	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
37 °C (aerobic)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Charcoal cefoperazone deoxycholate agar	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Glycine (1%)	V	–	–	V	+	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Resistance to:																																	
Nalidixic acid (30 µg)	–	–	–	V	–	+	+	V	+	+	+	V	V	–	V	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cefalotin (30 µg)	–	–	+	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
H <sub>2</sub> requirement	–	–	V	–	–	+	–	–	+	–	–	–	–	–	–	–	V	V	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
DNA G+C content (mol%)	30	31	35	ND	31	37–41	32	32	45–46	33–35	33	33–34	44–46	34	32–33	35–36	31–33	36	ND	31	30–31	36	30	29–30	36–38	29	45–46	44–46	29–30	32–36	28–30	29	29

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

subsp. nov. is proposed. Strains originating from phocid seals in Scotland form another subspecies, for which the name *C. pinnipeditorum* subsp. *caledonicus* subsp. nov. is proposed.

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

*Campylobacter pinnipeditorum* (pin.ni.pe.di.o'rum. N.L. gen. pl. n. *pinnipeditorum* pertaining to Pinnipedia).

Gram-reaction-negative, slightly curved to spiral-shaped rods. After incubation on Columbia agar with 5 % sheep blood in a microaerobic atmosphere at 37 °C for 72 h, colonies appear transparent to beige, glossy, slightly raised and circular with smooth margins. A clear dimorphic growth is observed: the majority of colonies are small (<0.5 mm), flat and appear transparent to beige; however, a minority form larger (0.5–1 mm), slightly raised, whitish and translucent colonies, which show  $\alpha$ -haemolysis. After 1 week of growth at 37 °C in a microaerobic atmosphere, colonies are 2–3 mm and appear circular with smooth margins, whitish, translucent and with a greenish periphery due to  $\alpha$ -haemolysis; however, in an anaerobic atmosphere, colonies appear circular with smooth margins, transparent with a whitish centre and radiating from the centre, while  $\alpha$ -haemolysis is absent. Shows no growth under aerobic conditions. No H<sub>2</sub> is required for growth under microaerobic conditions. In a microaerobic atmosphere, growth is observed after 48 h at 25 and 37 °C, but not at room temperature (18–22 °C) or 42 °C. All strains produce H<sub>2</sub>S on TSI agar and are positive for urea hydrolysis, oxidase activity and nitrate reduction, but are negative for hydrolysis of hippurate and indoxyl acetate. Catalase activity is variable. In a microaerobic atmosphere at 37 °C, normal growth is observed on Skirrow agar, but no growth is observed on charcoal cefoperazone deoxycholate agar and no or limited growth is observed on IST agar, Mueller–Hinton agar or in the presence of 1 % glycine. All strains are susceptible to cefalotin and nalidixic acid. Pathogenicity is unknown, although an association with infection is observed, as most currently known isolates have been recovered from abscesses and internal organs of pinnipeds.

The species type strain is RM17260<sup>T</sup> (=LMG 29472<sup>T</sup> =CCUG 69570<sup>T</sup>), which was isolated from an abscess of a California sea lion (*Zalophus californianus*) in 2013.

## DESCRIPTION OF *CAMPYLOBACTER PINNIPEDITORUM* SUBSP. *PINNIPEDITORUM* SUBSP. NOV.

*Campylobacter pinnipeditorum* subsp. *pinnipeditorum* (pin.ni.pe.di.o'rum. N.L. gen. pl. n. *pinnipeditorum* pertaining to Pinnipedia).

The strains adhere to the species description as given above. This subspecies can be distinguished from *Campylobacter pinnipeditorum* subsp. *caledonicus* subsp. nov. by divergent AtpA sequence and production of catalase. Pathogenicity is

unknown, although an association with infection is observed, as most currently known isolates have been recovered from abscesses and internal organs of California sea lions (*Zalophus californianus*) and a Steller sea lion (*Eumetopias jubatus*).

The subspecies type strain is RM17260<sup>T</sup> (=LMG 29472<sup>T</sup> =CCUG 69570<sup>T</sup>), which was isolated from an abscess of a California sea lion (*Zalophus californianus*) in 2013.

## DESCRIPTION OF *CAMPYLOBACTER PINNIPEDITORUM* SUBSP. *CALEDONICUS* SUBSP. NOV.

*Campylobacter pinnipeditorum* subsp. *caledonicus* [ca.le.do'ni.cus. L. masc. adj. *caledonicus* from Caledonia (Scotland), the geographical area from where the organism has been isolated].

The strains adhere to the species description as given above. This subspecies can be distinguished from *Campylobacter pinnipeditorum* subsp. *pinnipeditorum* by divergent AtpA sequence and the lack of catalase activity. Pathogenicity is unknown, although an association with infection is observed, as all currently known isolates have been recovered from abscesses of common seals (*Phoca vitulina*) or grey seal (*Halichoerus grypus*).

The subspecies type strain is M302/10/6<sup>T</sup> (=LMG 29473<sup>T</sup> =CCUG 68650<sup>T</sup>), which was isolated from a lung abscess of a grey seal (*Halichoerus grypus*) in 2010.

### Funding information

The Scottish Marine Animal Stranding Scheme receives financial support from the Scottish Government Marine Directorate and the UK Department of Environment, Farming and Rural Affairs (Defra). This work was also funded in part by the United States Department of Agriculture, Agricultural Research Service, CRIS projects 2030-42000-230-047 and 2030-42000-230-051.

### Acknowledgements

We thank Erika Nilson, Lexi Mena, Dr Todd Schmitt and the SeaWorld San Diego Stranded animal and laboratory teams for collecting and processing samples. We are grateful to Dr Barbara Byrne for providing strains. We thank Dr Aldert Zomer for support in sequencing. We also thank Dr James Bono for the PacBio sequencing of strains RM17260–RM17262. We are grateful to Tony Patterson, Andrew Brownlow and Bob Reid for providing material from post mortems on common and grey seals.

### Conflicts of interest

The authors declare that there are no conflicts of interest.

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