

Scotland's Rural College

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

Gilbert, MJ; Miller, WG; St. Leger, J; Chapman, MH; Timmerman, AJ; Duim, B; Foster, G; Wagenaar, JA

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1 **Title**

2 *Campylobacter pinnipediorum* sp. nov., isolated from pinnipeds, comprising *Campylobacter*  
3 *pinnipediorum* subsp. *pinnipediorum* subsp. nov. and *Campylobacter pinnipediorum* subsp.  
4 *caledonicus* subsp. nov.

5

6 **Running title**

7 *Campylobacter pinnipediorum* sp. nov.

8

9 **Contents category**

10 New taxa (Proteobacteria)

11

12 Maarten J. Gilbert<sup>1\*</sup>, William G. Miller<sup>2</sup>, Judy St. Leger<sup>3</sup>, Mary H. Chapman<sup>2</sup>, Arjen J.  
13 Timmerman<sup>1</sup>, Birgitta Duim<sup>1,5</sup>, Geoffrey Foster<sup>4</sup> and Jaap A. Wagenaar<sup>1,5,6</sup>

14

15 <sup>1</sup> Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine,  
16 Utrecht University, Utrecht, the Netherlands

17 <sup>2</sup> Produce Safety and Microbiology Research Unit, Agricultural Research Service, United  
18 States Department of Agriculture, Albany, California, United States

19 <sup>3</sup> SeaWorld Parks and Entertainment, San Diego, California, United States

20 <sup>4</sup> SAC Consulting Veterinary Services, Inverness, United Kingdom

21 <sup>5</sup> WHO Collaborating Center for *Campylobacter*/OIE Reference Laboratory for  
22 *Campylobacteriosis*, Utrecht, the Netherlands

23 <sup>6</sup> Wageningen Bioveterinary Research, Lelystad, the Netherlands

24

25 \* Corresponding author:

26 Email: m.j.gilbert@uu.nl. Phone: +31302534979.

27

28 The GenBank accession numbers for the whole genome sequences of strains RM17260,

29 RM17261, RM17262, M203/00/3, M302/10/6 and M341/11/05 are CP012546, CP012547,

30 CP012548, MBGA00000000, CP017018 and CP017258, respectively.

## 31 **Summary**

32 During independent diagnostic screenings of otariid seals in California (US) and phocid seals  
33 in Scotland (UK), *Campylobacter*-like isolates, which differed from the established  
34 *Campylobacter* taxa, were cultured from abscesses and internal organs of different seal  
35 species. A polyphasic study was undertaken to determine the taxonomic position of these six  
36 isolates. The strains were characterized by 16S rRNA and AtpA sequence analysis and by  
37 conventional phenotypic testing. The whole genome sequences were determined for all  
38 isolates and the average nucleotide identity (ANI) was determined. The isolates formed a  
39 separate phylogenetic clade, divergent from all other *Campylobacter* taxa and most closely  
40 related to *C. mucosalis*. Although all isolates showed 100% 16S rRNA sequence homology,  
41 AtpA and ANI analyses indicated divergence between the otariid isolates from California and  
42 the phocid isolates from Scotland, which warrants subspecies status for each clade. The two  
43 subspecies can also be distinguished phenotypically based on catalase activity. This study  
44 shows clearly that the isolates obtained from pinnipeds represent a novel species within the  
45 genus *Campylobacter*, for which the name *Campylobacter pinnipediorum* sp. nov. is  
46 proposed. Within this novel species, the Californian isolates represent a separate subspecies,  
47 for which the name *C. pinnipediorum* subsp. *pinnipediorum* subsp. nov. is proposed. The type  
48 strain for both this novel species and subspecies is RM17260<sup>T</sup> (= LMG 29472<sup>T</sup> = CCUG  
49 69570<sup>T</sup>). The Scottish isolates represent another subspecies, for which the name *C.*  
50 *pinnipediorum* subsp. *caledonicus* subsp. nov. is proposed. The type strain of this subspecies  
51 is M302/10/6<sup>T</sup> (= LMG 29473<sup>T</sup> = CCUG 68650<sup>T</sup>).

52 Of all currently recognized *Campylobacter* species, at least five are predominantly associated  
53 with animals found in marine environments. *Campylobacter lari*, *C. peloridis*, *C.*  
54 *subantarcticus* and *C. volucris* (Debruyne *et al.*, 2009, Debruyne *et al.*, 2010a, Debruyne *et*  
55 *al.*, 2010b) are all isolated from shellfish and/or marine birds, whereas *C. insulaenigrae* has  
56 been isolated from marine mammals (Foster *et al.*, 2004). These predominantly  
57 thermotolerant species are all closely related and belong to the same clade, which includes  
58 the human pathogen *C. jejuni*. In contrast, *Campylobacter* species from the clade to which *C.*  
59 *concisus* and *C. mucosalis* belong are not or are rarely associated with marine animals; many  
60 are isolated from the oral cavity of terrestrial vertebrates, including humans (Debruyne *et al.*,  
61 2008). We describe a novel, urease positive *Campylobacter* species, related to *C. mucosalis*  
62 and *C. concisus*, which has been isolated from pinnipeds. Furthermore, this species is  
63 proposed to contain two subspecies.

64

65 Pinnipeds comprise the families Odobenidae (walruses), Otariidae (eared seals) and Phocidae  
66 (earless seals). Six *Campylobacter*-like isolates not belonging to any of the established  
67 *Campylobacter* taxa were obtained from pinnipeds during independent diagnostic screenings  
68 in California (United States) and Scotland (United Kingdom). Three isolates were obtained  
69 from internal organs and an abscess of California sea lion (*Zalophus californianus*) juveniles  
70 from a seal rehabilitation centre in San Diego. Three isolates were obtained from abscesses of  
71 stranded deceased common seal (*Phoca vitulina*) and grey seal (*Halichoerus grypus*)  
72 juveniles from the coastal regions of Scotland. Noteworthy, from two of these abscesses no  
73 organisms other than the *Campylobacter*-like organisms were isolated.

74

75 Initial characterization using AFLP (Duum *et al.*, 1999) and 16S rRNA sequencing (Maiwald,  
76 2004) indicated that these isolates were different from all other *Campylobacter* species, but

77 most closely related to *C. mucosalis*. A recent study analysing the microbiota of sea  
78 mammals (Bik *et al.*, 2016) identified *Campylobacter* 16S rRNA sequences in both oral and  
79 gastric samples from California sea lions, but not from common bottlenose dolphins  
80 (*Tursiops truncatus*). These 16S rRNA sequences shared 99-100% sequence homology with  
81 the 16S rRNA sequences of the isolates described in this study.

82

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

88

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

95

96 Complete genome sequences for strains RM17260, RM17261, RM17262 and M302/10/6  
97 were obtained as described (Miller *et al.*, manuscript in preparation). Briefly, initial  
98 sequencing was performed on a Roche 454 GS-FLX+ Genome Sequencer (Roche Life  
99 Science, Indianapolis, IN). 454 sequencing reads were assembled into single scaffolds using  
100 the Roche Newbler assembler (ver. 2.6) and base calls were validated using Illumina MiSeq  
101 (Illumina Inc., San Diego, CA) reads. Additional sequencing was performed for the above  
102 four strains and de novo for strains M341/11/05 and M203/00/3 using a PacBio RS sequencer  
103 (Pacific Biosciences, Menlo Park, CA) to generate complete, closed genomes. Draft genomes  
104 for strains RM18812, RM18813, RM18906, 1105248A and 03036546 were obtained using  
105 the Illumina MiSeq. Sequencing reads were assembled using Newbler or SPAdes (ver. 3.1.1).  
106 The genome sequences have been deposited in GenBank; accession numbers are listed in  
107 Table 1.

108  
109 The taxonomic position of all strains was determined by 16S rRNA gene comparison. The  
110 16S rRNA gene sequences ( $\geq 1339$  bp) were extracted from the whole genome sequences of  
111 the strains or obtained from EzTaxon (Kim *et al.*, 2012) for the other *Campylobacter* species.  
112 Sequence alignment and dendrogram construction were performed using CLUSTALX (ver.  
113 2.1) and MEGA version 6.05 (Tamura *et al.*, 2013). A neighbour-joining dendrogram  
114 containing all *Campylobacter* taxa was constructed (Fig. 1). Bootstrap values were  
115 determined using 500 repetitions. The 16S rRNA gene sequence from *Arcobacter butzleri*  
116 strain RM4018 was used to root the tree. The 16S rRNA gene sequence similarity between  
117 the pinniped-associated strains was 100%, while the sequence similarity between these strains  
118 and the most closely related species *C. concisus* and *C. mucosalis* was 96-97%.

119  
120 For improved taxonomic resolution (Miller *et al.*, 2014a), full AtpA protein sequences were

121 extracted from the whole genome sequences or obtained from GenBank. Alignment and  
122 dendrogram construction were performed as described above; the AtpA sequence from  
123 *Arcobacter butzleri* strain RM4018 was used to root the tree. Consistent with the 16S rRNA  
124 comparison, the pinniped-associated strains formed a clade distinct from other  
125 *Campylobacter* taxa (Fig. 2). Furthermore, a clear distinction could be observed between the  
126 strains isolated in California and Scotland.

127

128 As an alternative for DNA-DNA hybridization (DDH), the average nucleotide identity (ANI)  
129 has been suggested (Konstantinidis & Tiedje, 2005, Konstantinidis *et al.*, 2006). A DDH  
130 species delineation of 70% corresponds to about 95% ANI (Goris *et al.*, 2007). Using the  
131 JSpecies v. 1.2.1 (Richter & Rosselló-Móra, 2009), pair-wise ANI values based on whole  
132 genome sequences were calculated for all the unidentified *Campylobacter* strains and the  
133 most closely related species: *C. concisus* (strain 13826; accession no. CP000792), *C. curvus*  
134 (strain 525.92; accession no. CP000767), *C. mucosalis* (strain DSM 21682<sup>T</sup>; accession no.  
135 JHQQ01), *C. rectus* (strain ATCC 33238<sup>T</sup>; accession no. ACFU00000000) and *C. showae*  
136 (strain ATCC 51146<sup>T</sup>; accession no. ACVQ00000000). While ANI for digital DDH  
137 assessments of *Campylobacter* and related species has not been tested, the ANI between the  
138 pinniped-associated strains and most closely related species (*C. mucosalis*) was maximally  
139 71%, which is well below the 95% species cut-off suggested by Goris and colleagues (2007)  
140 and similar to ANI values observed between the pinniped-associated strains and the other  
141 related species (Table 2). Strains originating from either California or Scotland were highly  
142 homologous amongst each other (ANI  $\geq$  98%). However, 94-95% ANI was observed  
143 between the Californian and Scottish strains, indicating divergence between both groups of  
144 strains on a genomic level. The taxonomic position of the novel *Campylobacter* strains is



145 further supported by a core genome phylogeny which includes these strains and related  
146 *Campylobacter* taxa (Miller *et al.*, manuscript in preparation).

147

148 The genetic analyses presented here indicate that the pinniped-associated strains form a  
149 distinct clade which is clearly separated from the closest known relatives. Based on the 100%  
150 16S rRNA homology, all strains examined clearly belong to the same species. However, the  
151 divergence observed between the Californian and Scottish strains, based on the ANI, AtpA  
152 and core genome phylogeny, warrants subspecies status for each group. The 94-95% ANI  
153 observed between the Californian and Scottish strains is consistent with the subspecies  
154 divergence observed in other *Campylobacter* species, such as *C. fetus* (8% divergence  
155 between *C. fetus* subsp. *fetus* and *C. fetus* subsp. *testudinum*) and *C. hyointestinalis* (6%  
156 divergence between *C. hyointestinalis* subsp. *hyointestinalis* and *C. hyointestinalis* subsp.  
157 *lawsonii*) (Miller *et al.*, 2016).

158

159 The G+C content was determined based on the whole genome sequences using Artemis  
160 v.13.2 (Wellcome Trust Sanger Institute, UK; Rutherford *et al.*, 2000). All strains had a G+C  
161 content varying between 30.4% and 31.0%, which is within the range observed in  
162 *Campylobacter* (Table 3).

163

164 Additional phenotypic testing was performed as described previously (On & Holmes, 1991a,  
165 On & Holmes, 1991b, On & Holmes, 1992, Ursing *et al.*, 1994). Oxidase activity, catalase  
166 activity, nitrate reduction, indoxyl acetate hydrolysis, urea hydrolysis, hippurate hydrolysis  
167 and H<sub>2</sub>S production on TSI agar were determined. In addition to this, growth with 1%  
168 glycine,  $\alpha$ -haemolysis on sheep blood agar, H<sub>2</sub> requirement and resistance to nalidixic acid  
169 (30  $\mu$ g) and cephalothin (30  $\mu$ g) were evaluated. Strains were grown at various temperatures,

170 atmospheres and on different agar media. The strains displayed phenotypic characters distinct  
171 from all other *Campylobacter* taxa. All strains displayed urease activity, which may be  
172 related to a gastric niche. Indeed, 99-100% 16S rRNA sequence homology was observed  
173 between the strains and uncultured bacteria from the gastric microbiota of California sea lions  
174 (Bik *et al.*, 2016). Differentiating characteristics for the strains tested and other  
175 *Campylobacter* taxa are summarized in Table 3. Based on urease activity, H<sub>2</sub>S production on  
176 TSI agar, nitrate reduction, growth at 25°C in a microaerobic atmosphere and  $\alpha$ -haemolysis,  
177 the strains can be distinguished from all other described *Campylobacter* taxa. Results of the  
178 discriminatory phenotypic tests were identical for the five additional *Campylobacter* strains.  
179 Catalase activity was observed in all strains originating from otariid seals in California, but  
180 not in strains originating from phocid seals in Scotland, supporting the existence of two  
181 subspecies. Urease-positive *Campylobacter lari* (UPTC) are not, as yet, a defined taxon  
182 within *Campylobacter* (Bolton *et al.*, 1985, Megraud *et al.*, 1988, Endtz *et al.*, 1997,  
183 Debruyne *et al.*, 2009). Nevertheless, since such strains are also urease positive they could  
184 potentially share the same phenotypic profile as the pinniped-associated strains. Therefore,  
185 their phenotypic characteristics were also analysed, based on *C. lari* strains NCTC 11845,  
186 CCUG 22395, RM16701 and RM16712 (Miller *et al.*, 2014b). UPTC strains could not grow  
187 at 25°C microaerobically and did not produce H<sub>2</sub>S on TSI agar; thus, the pinniped-associated  
188 strains could also be readily distinguished from the urease positive *C. lari*.

189 In conclusion, the results from this polyphasic taxonomic study clearly demonstrate that the  
190 isolates recovered from pinnipeds comprise a novel species distinct from all other currently  
191 known *Campylobacter* species, based on 16S rRNA, AtpA, whole genome sequence  
192 comparison and biochemical properties. The name *Campylobacter pinnipediorum* sp. nov. is  
193 proposed for these strains. Within this novel species, strains originating from otariid seals in  
194 California form a separate subspecies, for which the name *C. pinnipediorum* subsp.  
195 *pinnipediorum* subsp. nov. is proposed. Strains originating from phocid seals in Scotland  
196 form another subspecies, for which the name *C. pinnipediorum* subsp. *caledonicus* subsp.  
197 nov. is proposed.

198 **Description of *Campylobacter pinnipediorum* sp. nov.**

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

201

202 Gram-negative slightly curved to spiral-shaped rods. After incubation on Columbia agar with  
203 5% sheep blood in a microaerobic atmosphere at 37°C for 72 h colonies appear transparent to  
204 beige, glossy, slightly raised and circular with smooth margins. A clear dimorphic growth  
205 was observed: the majority of colonies are small (< 0.5 mm), flat and appear transparent to  
206 beige; however, a minority formed larger (0.5-1 mm), slightly raised, whitish and translucent  
207 colonies, which show  $\alpha$ -haemolysis. After a week of growth at 37°C in a microaerobic  
208 atmosphere colonies are 2-3 mm and appear circular with smooth margins, whitish,  
209 translucent, with greenish periphery due to  $\alpha$ -haemolysis; however, in an anaerobic  
210 atmosphere colonies appear circular with smooth margins, transparent with a whitish centre  
211 and radiating from the centre, while  $\alpha$ -haemolysis is absent. Shows no growth at aerobic  
212 conditions. No H<sub>2</sub> is required for growth at microaerobic conditions. In a microaerobic  
213 atmosphere, growth is observed after 48 h at 25°C and 37°C, but not at room temperature  
214 (18-22°C) or 42°C. All strains produced H<sub>2</sub>S on TSI agar and were positive for urea  
215 hydrolysis, oxidase activity and nitrate reduction, but were negative for hydrolysis of  
216 hippurate and indoxyl acetate. Catalase activity is variable. In a microaerobic atmosphere at  
217 37°C normal growth on Skirrow agar, but no growth on charcoal cefoperazone deoxycholate  
218 (CCD) agar and no or limited growth on IST agar, Mueller-Hinton agar nor in the presence of  
219 1% glycine. All strains were susceptible to cephalothin and nalidixic acid. Pathogenicity is  
220 unknown, although an association with infection is observed, as most currently known  
221 isolates have been recovered from abscesses and internal organs of pinnipeds. The species

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

224

225 **Description of *Campylobacter pinnipediorum* sp. nov. subsp. *pinnipediorum* subsp. nov.**

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

228

229 The strains adhere to the species description as given above. This subspecies can be  
230 distinguished from *Campylobacter pinnipediorum* sp. nov. subsp. *caledonicus* subsp. nov. by  
231 divergent AtpA sequence and production of catalase. Pathogenicity is unknown, although an  
232 association with infection is observed, as most currently known isolates have been recovered  
233 from abscesses and internal organs of California sea lions (*Zalophus californianus*) and a  
234 Steller sea lion (*Eumetopias jubatus*). The subspecies type strain is RM17260<sup>T</sup> (= LMG  
235 29472<sup>T</sup> = CCUG 69570<sup>T</sup>), which was isolated from an abscess of a California sea lion  
236 (*Zalophus californianus*) in 2013.

237

238 **Description of *Campylobacter pinnipediorum* sp. nov. subsp. *caledonicus* subsp. nov.**

239 *Campylobacter pinnipediorum* subsp. *caledonicus* (ca.le.do'ni.cus. L. masc. adj. caledonicus,  
240 from Caledonia (Scotland), the geographic area where the organism has been isolated).

241

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

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

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344 **Figure legends & tables**

345

346 Figure 1. Neighbor-joining phylogenetic dendrogram based on 16S rRNA gene sequences.

347 Bootstrap values ( $\geq 75\%$ ) based on 500 replications are indicated at the nodes.

348

349 Figure 2. Neighbor-joining phylogenetic dendrogram based on AtpA protein sequences.

350 Bootstrap values ( $\geq 75\%$ ) based on 500 replications are indicated at the nodes. *C.*

351 *pinnipediorum* genome sequence accession numbers are as listed in Fig. 1.

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

353

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

354

355 Table 2. Average nucleotide identity (ANI) values (%) based on BLAST for *C.*

356 *pinnipediorum* sp. nov. and the most closely-related *Campylobacter* species.

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

357

358 Strains: 1, *C. pinnipediorum* sp. nov. RM17260<sup>T</sup>; 2, *C. pinnipediorum* sp. nov. RM17261; 3,

359 *C. pinnipediorum* sp. nov. RM17262; 4, *C. pinnipediorum* sp. nov. RM18812; 5, *C.*

360 *pinnipediorum* sp. nov. RM18813; 6, *C. pinnipediorum* sp. nov. RM18906; 7, *C.*

361 *pinnipediorum* sp. nov. 1105248A; 8, *C. pinnipediorum* sp. nov. 0306546; 9, *C.*

362 *pinnipediorum* sp. nov. M302/10/6; 10, *C. pinnipediorum* sp. nov. M341/11/05; 11, *C.*

363 *pinnipediorum* sp. nov. M203/00/3; 12, *C. concisus* 13826; 13, *C. curvus* 525.92; 14, *C.*

364 *mucosalis* ATCC 49352<sup>T</sup>; 15, *C. rectus* ATCC 33238<sup>T</sup>; 16, *C. showae* ATCC 51146<sup>T</sup>. Strains

365 1-8, *C. pinnipediorum* subsp. *pinnipediorum* subsp. nov.; strains 9-11, *C. pinnipediorum*

366 subsp. *caledonicus* subsp. nov.

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

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	+	+	+	+	+	V	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	V	+	+	+	+	+		
Catalase	+	-	+	V	+	-	+	+	-	+	+	(+)	(-)	-	-	+	+	+	+	V	+	+	+	+	-	+	(-)	+	V*	+	-	V	+		
Urease	+	+	-	V	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	-	-	V*	ND	-	+	ND	
Nitrate reduction	+	+	+	V	+	(-)	(+)	+	+	+	+	(+)	(+)	+	-	+	+	+	+	-	+	+	+	+	(-)	ND	+	+	(+)	+	+	+	+		
Hippurate hydrolysis	-	-	+	-	-	-	-	-	(-)	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-		
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	
H <sub>2</sub> S 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	-
25°C (microaerobic)	+	+	-	-	-	-	ND	-	-	+	+	+	-	-	-	(-)	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37°C (microaerobic)	+	+	+	+	+	+	+	+	V	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	V	+	+	+	+	+	
42°C (microaerobic)	-	-	+	+	+	(+)	ND	(+)	V	(+)	+	-	V	+	(-)	+	+	-	-	-	+	+	+	+	+	+	+	(-)	V	+	+	+	+	V	+
37°C (anaerobic)	+	+	-	+	-	+	+	-	+	(-)	+	V	+	-	+	-	+	+	-	-	-	+	ND	-	+	ND	+	+	+	+	+	+	-	+	+
37°C (aerobic)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CCDA	-	-	-	+	+	(-)		(+)	(+)	+	+	+	V	+	ND	+	+	+	ND	+	+	ND	+	+	+	+	-	+	(+)	ND	+	ND	ND	ND	
Glycine (1%)	V	-	-	V	(+)	(-)	+	-	+	+	+	(-)	+	V	+	+	V	+	+	(-)	+	-	(+)	+	(-)	+	+	V	+	(+)	+	+	+	-	
Resistance to:																																			
Nalidixic acid (30 µg)	-	-	-	V	-	(+)	+	V	+	+	+	V	V	-	V	+	+	+	+	-	-	+	-	(+)	(+)	(+)	(+)	-	(+)	+	-	-	-	+	
Cephalothin (30 µg)	-	-	+	-	+	-	-	(+)	-	-	ND	-	-	-	(-)	-	-	+	-	+	+	+	+	+	-	(-)	-	-	-	-	(-)	-	-	+	
H <sub>2</sub> requirement	-	-	V	-	-	+	-	-	+	-	ND	-	+	-	+	V	V	-	ND	-	-	-	ND	-	+	ND	+	+	-	ND	-	+	ND	ND	
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	29-33	30	32-36	28-30	29		

369 Taxa: 1, *C. pinnipediorum* subsp. *pinnipediorum* subsp. nov. ( $n = 3$ ); 2, *C. pinnipediorum*  
370 subsp. *caledonicus* subsp. nov. ( $n = 3$ ); 3, *C. avium*; 4, *C. canadensis*; 5, *C. coli*; 6, *C.*  
371 *concisus*; 7, *C. corcagiensis*; 8, *C. cuniculorum*; 9, *C. curvus*; 10, *C. fetus* subsp. *fetus*; 11, *C.*  
372 *fetus* subsp. *testudinum*; 12, *C. fetus* subsp. *venerealis*; 13, *C. gracilis*; 14, *C. helveticus*; 15,  
373 *C. hominis*; 16, *C. hyointestinalis* subsp. *hyointestinalis*; 17, *C. hyointestinalis* subsp.  
374 *lawsonii*; 18, *C. iguaniorum*; 19, *C. insulaenigrae*; 20, *C. jejuni* subsp. *doylei*; 21, *C. jejuni*  
375 subsp. *jejuni*; 22, *C. lanienae*; 23, *C. lari* subsp. *concheus*; 24, *C. lari* subsp. *lari*; 25, *C.*  
376 *mucosalis*; 26, *C. peloridis*; 27, *C. rectus*; 28, *C. showae*; 29, *C. sputorum*; 30,  
377 *C. subantarcticus*; 31, *C. upsaliensis*; 32, *C. ureolyticus*; 33, *C. volucris*. Characteristics of  
378 reference taxa were adapted from previous species descriptions (Vandamme *et al.*, 2010,  
379 Koziel *et al.*, 2014, Gilbert *et al.*, 2015). +, 90-100%; (+), 75-89%; V, 26-74%; (-), 11-25%; -  
380 , 0-10%; ND, not determined; \*, test results differ between *C. sputorum* biovars *sputorum*  
381 (catalase and urease negative), *paraureolyticus* (catalase negative, urease positive) and *fecalis*  
382 (catalase positive, urease negative).