Campylobacter canadensis sp. nov., from captive whooping cranes in Canada

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Ten isolates of an unknown *Campylobacter* species were isolated from cloacal swabs obtained from captive adult whooping cranes (*Grus americana*). All isolates were identified as *Campylobacter* based on generic PCR and grouped with other *Campylobacter* species based on 23S rRNA gene sequence. None of the isolates could be identified by species-specific PCR for known taxa, and all ten isolates formed a robust clade that was very distinct from known *Campylobacter* species based on 16S rRNA, *rpoB* and *cpn60* gene sequences. The results of 16S rRNA gene nucleotide sequence ($\leqslant$92 % sequence similarity to recognized *Campylobacter* species) and genomic DNA (no detectable relatedness) analyses were consistent with novel species status. Cells of the *Campylobacter* from whooping cranes were uniflagellar and typically sigmoid to allantoid in shape (0.48 μm wide and 2.61 μm long), but also spheroid to coccoid (0.59 μm wide and 0.73 μm long). The bacterium was oxidase-positive, able to reduce nitrite, able to grow at 37° and 42 °C, and grew anaerobically, as well as in an atmosphere devoid of H_2, and on MacConkey agar. It was not α-haemolytic and was negative for hippurate and indoxyl acetate hydrolysis and alkaline phosphatase. It also was susceptible to cephalotin and was unable to grow on nutrient agar, on a medium containing 3.5 % NaCl or in ambient O_2. The bacterium was unable to grow at 25 °C and growth was negative or very restricted at 30 °C. Fluorescent amplified fragment length polymorphism analysis indicated that nine of the recovered isolates were genetically distinct. A species-specific primer set targeting the *cpn60* gene was developed. The name *Campylobacter canadensis* sp. nov. is proposed for the novel species, with the type strain L266^T (=CCUG 54429^T =LMG 24001^T).

Ten isolates of a *Campylobacter*-like bacterium were recovered from captive whooping cranes (*Grus americana*), a bird whose populations have been so decimated by human activities that the species is currently classified as endangered in both Canada and the USA. In this paper, we describe the cultural and biochemical characteristics of this bacterium and the results of a polyphasic taxonomic investigation.

Three unidentified isolates of a *Campylobacter*-like bacterium were recovered from cloacae of three whooping cranes in 2004 during a study to characterize the normal enteric bacterial flora of captive whooping and sandhill cranes at the Calgary Zoo (Hoar *et al.*, 2007). Seven additional isolates were recovered from whooping cranes in 2006. Isolations were made on a *Campylobacter*-selective Preston medium containing selective supplement SR117 (Oxoid), Karmali agar containing selective supplement SR167 (Oxoid) or *Campylobacter* charcoal-deoxycholate medium (CCDA) containing selective supplement SR155 (Oxoid) incubated anaerobically (10 % CO_2, 10 % H_2 and 80 % N_2) or microaerobically (5 % O_2, 10 % CO_2, 3 % H_2

Abbreviation: AFLP, amplified fragment length polymorphism.

The GenBank/EMBL/DDBJ accession numbers for the 23S rRNA, 16S rRNA, *rpoB* and *cpn60* gene sequences of the novel isolates determined in this study are EF621904 and EF621905 (23S rRNA), EF621894–EF621903 (16S rRNA), EF621885–EF621893 (*rpoB*) and EF621906–EF621915 (*cpn60*), as detailed in Figs 2–5; the accession numbers for strain L266^T are respectively EF621904, EF621894, EF621885 and EF621906.

and 83 % N_2) at 37 or 42 °C. Cultures were examined after 2, 3 and 7 days incubation. The physiological characters of all ten isolates along with selected reference strains were determined using standard methods (e.g. On *et al.*, 1996 and references therein); all tests were conducted at least two times. Morphological characteristics of the bacterium were determined using light and transmission electron microscopy (TEM). For TEM, cells (48 h old) were fixed in 2 % glutaraldehyde, stained with uranyl acetate and examined with a Hitachi H-7100 microscope operated at 30 kV.

Genomic DNA was extracted using the Qiagen DNeasy tissue kit. Extracted genomic DNA was subjected to genus *Campylobacter*- and *Campylobacter* species-specific PCR (Inglis & Kalischuk, 2003; Lawson *et al.*, 2001). The 23S rRNA genes of two isolates, L266^T and L268, were amplified using the basic primers and PCR conditions published by Sallen *et al.* (1996) with slight modifications to the primer sequences (Inglis *et al.*, 2006). The 16S rRNA gene was amplified using the eubacterial primers UNI27F and UNI1492R (Lane, 1991), and sequences were obtained using the primers UNI27F, UNI338F, UNI1100R and UNI1492R. The *rpoB* and *cpn60* genes (synonyms *groEL* and *hsp60*) were sequenced as described by Korczak *et al.* (2006) and Inglis *et al.* (2006), respectively. All sequences were compared directly with the NCBI non-redundant nucleotide database using BLASTN. Subsequently, the nucleotide sequences for the unidentified isolates were aligned along with data retrieved from NCBI using the multialignment program CLUSTAL W, and the alignments were refined visually using GeneDoc (Nicholas & Nicholas, 1997). Sequence data were analysed using programs contained within the phylogenetic software PHYLIP (Felsenstein, 2005). Phylogenetic estimates were obtained based on the neighbour-joining distance method. Divergence (or distance) of each pair of sequences was calculated by DNADIST using Kimura's two-parameter model. The NEIGHBOR program was used to carry out the neighbour-joining method for estimating phylogenies from the distance matrices. Support for internal branches within the resulting trees was obtained by bootstrap analysis (1000 replicates) generated by SEQBOOT, and a majority-rule consensus tree was constructed using the CONSENSE program. TreeView (Page, 2001) was used to generate rooted phylogenetic trees. Species-specific primers targeting the *cpn60* gene were developed using the COnsensus-DEgenerate Hybrid Oligonucleotide Primers (CODEHOP) program (http://bioinformatics.weizmann.ac.il/blocks/codehop.html). The primers developed were CraneF (5′-GAAAAAGTAGGCAAAGATGGTGTTA-3′) and CraneR (5′-CAATTATTAAAAGCGGTC-3′). The predicted annealing temperature was 46.5 °C, no hairpin loops or primer dimers were predicted and the projected amplicon was 253 bases long. Each primer was subjected to BLAST analysis using the NCBI website (short but nearly exact matches). The forward primer produced identical alignments to the *cpn60* genes of *Campylobacter jejuni*, *Campylobacter coli*, *Helicobacter pullorum*, *Helicobacter pametensis* and *Wolinella succinogenes*. In contrast, the reverse primer was predicted to be specific for the crane isolates. The PCR mixture (20 µl) consisted of 2 µl 10 × buffer, 0.4 µl 10 mM dNTPs, 0.4 µl 25 mM $MgCl_2$, 1.0 µl each of 10 µM solutions of CraneF and CraneR, 2.0 µl BSA, 0.1 µl Qiagen HotStar *Taq* polymerase (5 U μl^{-1}), 11.1 µl Optima water and 2 µl DNA (20–100 ng). PCR conditions applied were 15 min at 95 °C followed by 35 cycles of 30 s at 94 °C, 60 s at 47 °C and 60 s at 72 °C, followed by 10 min at 72 °C. For DNA–DNA hybridization, the method of Mehlen *et al.* (2004) was employed with DNA from L266^T and L284 used as the digoxigenin-labelled probes. The G+C content of reference strains was determined from Vandamme *et al.* (1991) and Logan *et al.* (2000). The clonality of the isolates was investigated by fluorescent amplified fragment length polymorphism (AFLP) analysis using the basic procedure described by

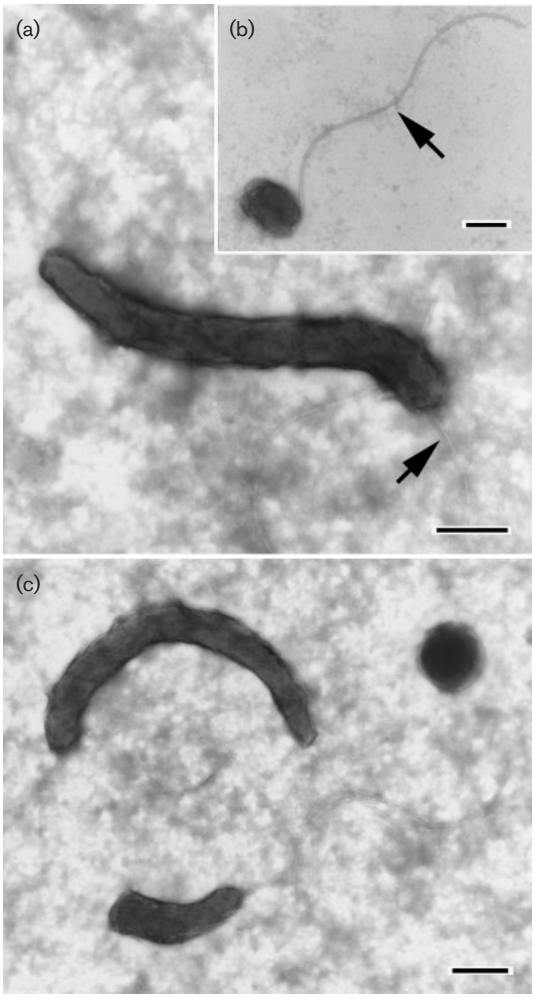

Fig. 1. Transmission electron micrographs of strain L267 (*Campylobacter canadensis* sp. nov.) showing the sigmoid form (a), coccoid form (b) and polymorphic cells (c). Bars, 500 nm. Note the presence of a single polar flagellum (arrows).

Table 1. Physiological characteristics of *Campylobacter* and *Arcobacter* species

Taxa: 1, *Campylobacter canadensis* sp. nov.; 2, *Arcobacter butzleri*; 3, *A. cryaerophilus*; 4, *A. nitrofigilis*; 5, *A. skirrowii*; 6, *Bacteroides ureolyticus* [species *incertae sedis* that is genotypically a member of *Campylobacter*; furthermore, Han *et al.* (1991) indicated that *B. ureolyticus* is a microaerophilic and not an anaerobic taxon]; 7, *C. coli*; 8, *C. concisus*; 9, *C. curvus*; 10, *C. fetus* subsp. *fetus*; 11, *C. fetus* subsp. *venerealis*; 12, *C. gracilis*; 13, *C. helveticus*; 14, *C. hominis*; 15, *C. hyointestinalis* subsp. *hyointestinalis*; 16, *C. hyointestinalis* subsp. *lawsonii*; 17, *C. insulaenigrae*; 18, *C. jejuni* subsp. *doylei*; 19, *C. jejuni* subsp. *jejuni*; 20, *C. lanienae*; 21, *C. lari*; 22, *C. mucosalis*; 23, *C. rectus*; 24, *C. showae*; 25, *C. sputorum*; 26, *C. upsaliensis*. Data for reference species were taken from Foster *et al.* (2004), Lawson *et al.* (2001), Logan *et al.* (2000) and On *et al.* (1996). +, 90–100 % of strains positive; (+), 75–89 % of strains positive; V, 26–74 % of strains positive; (−), 11–25 % of strains positive; −, 0–10 % of strains positive. NA, No data available.

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
α-Haemolysis	−	−	−	−	+	V	(−)	(−)	(−)	−	V	−	+	−	V	V	NA	+	+	−	V	−	+	+	+	+
Oxidase	+	+	+	+	+	+	+	V	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	V	+	+
Catalase	V	V	+	+	+	(−)	+	−	−	+	(+)	V	−	−	+	+	+	V	+	+	+	−	(−)	+	V	−
Hippurate hydrolysis	−	−	−	−	−	−	−	−	(−)	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−
Urease production	V	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	V	−	−	−	−	−
Nitrate reduction	V	+	+	+	+	+	+	(−)	+	+	+	(+)	+	V	+	+	+	−	+	+	+	−	+	+	+	+
Alkaline phosphatase	−	−	−	−	−	−	−	V	V	−	−	−	−	−	−	(−)	NA	−	−	+	−	(+)	−	−	−	−
Production on TSI agar of:																										
H_2S	V	−	−	−	−	−	V	−	(−)	−	−	−	−	−	+	+	−	−	−	−	−	+	−	V	+	−
Acid and H_2S	(−)	−	−	−	−	−	−	−	−	−	−	−	−	NA	−	−	NA	−	−	V	−	−	−	−	−	−
Indoxyl acetate hydrolysis	−	+	+	+	+	−	+	−	V	−	−	V	+	−	−	−	−	+	+	−	−	−	+	V	−	+
Growth at/in/on:																										
25 °C (microaerobic)	−	+	+	+	+	−	−	−	−	+	+	−	−	−	(−)	−	−	−	−	−	−	−	−	−	−	−
30 °C (microaerobic)	−	+	+	+	+	+	+	(+)	+	+	+	V	V	−	+	+	NA	+	+	NA	+	+	V	+	(+)	+
37 °C (microaerobic)	+	+	V	−	+	−	+	+	V	+	+	−	+	−	+	+	NA	+	+	+	+	+	−	V	+	+
42 °C (microaerobic)	+	(−)	−	−	(−)	V	+	(+)	V	(+)	−	V	+	NA	+	+	−	−	+	+	+	+	(−)	V	+	(+)
37 °C (anaerobic)	+	+	(−)	+	+	+	−	+	+	(−)	V	+	−	+	−	+	−	−	−	+	−	+	+	+	+	−
Ambient O_2	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Nutrient agar	−	+	+	+	+	+	+	(−)	+	+	+	+	(+)	NA	+	+	NA	+	+	NA	+	+	(−)	V	+	+
CCDA	+	+	+	−	+	V	+	(−)	(+)	+	+	V	+	NA	+	+	NA	+	+	NA	+	+	−	+	(+)	+
MacConkey agar	+	(+)	(−)	−	−	(−)	V	−	(+)	(+)	V	(+)	−	−	V	V	NA	−	−	+	−	(+)	−	+	V	−
1 % glycine	V	−	−	−	−	+	+	(−)	+	+	−	+	V	+	+	V	+	(−)	+	−	+	V	+	V	+	+
3.5 % NaCl	−	V	−	+	+	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	V	−
Resistance to nalidixic acid	V	(−)	−	−	−	−	−	(+)	+	+	V	V	−	V	+	+	+	−	−	+	V	(+)	(+)	−	(+)	−
Resistance to cephalotin	−	+	+	−	+	−	+	−	−	−	−	−	−	−	(−)	−	+	−	+	+	+	−	−	−	−	(−)
Requirement for H_2	−	NA	NA	NA	NA	NA	−	+	+	−	−	NA	−	NA	V	V	NA	−	−	−	−	+	+	+	+	−
γ-Glutamyl transpeptidase	(+)	NA	NA	NA	NA	NA	−	−	NA	−	NA	NA	−	NA	−	−	NA	NA	−	−	−	NA	NA	NA	−	−
Nitrite reduction	+	NA	NA	NA	NA	NA	−	−	NA	−	NA	NA	−	NA	−	−	−	NA	−	V	−	NA	NA	NA	−	−
Flagella	+	+	+	+	+	NA	+	+	+	+	+	−	+	−	+	+	NA	+	+	+	+	+	+	+	+	+

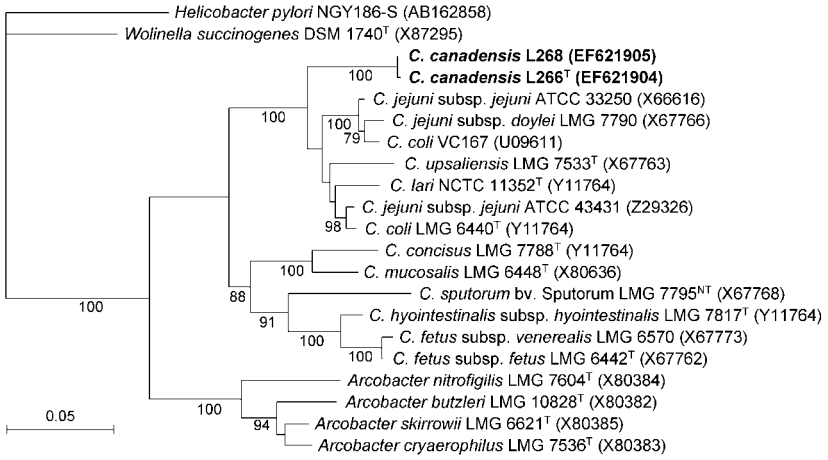

Fig. 2. Rooted tree based on 23S rRNA gene sequences, showing the phylogenetic relationships of two strains of *C. canadensis* sp. nov. NCBI accession numbers are presented in parentheses. Bar, 0.05 nucleotide substitutions per base. Numbers at nodes ($\geqslant$70 %) indicate support for the internal branches within the tree obtained by bootstrap analysis (percentages of 1000 bootstraps).

Kokotovic & On (1999). Amplified DNA products were separated using POP7 polymer with an ABI 3130 Automated Genetic Analyzer (Applied Biosystems). Only AFLP profiles comprising fragments detected in the size range 80 to 500 bp were considered for numerical analysis. GENESCAN-processed data files comprising both bacterial AFLP profiles and the internal molecular mass standard (GENESCAN-500 LIZ) were imported into the program BioNumerics 4.01 (Applied Maths). After registration of strain details, profiles were normalized within and

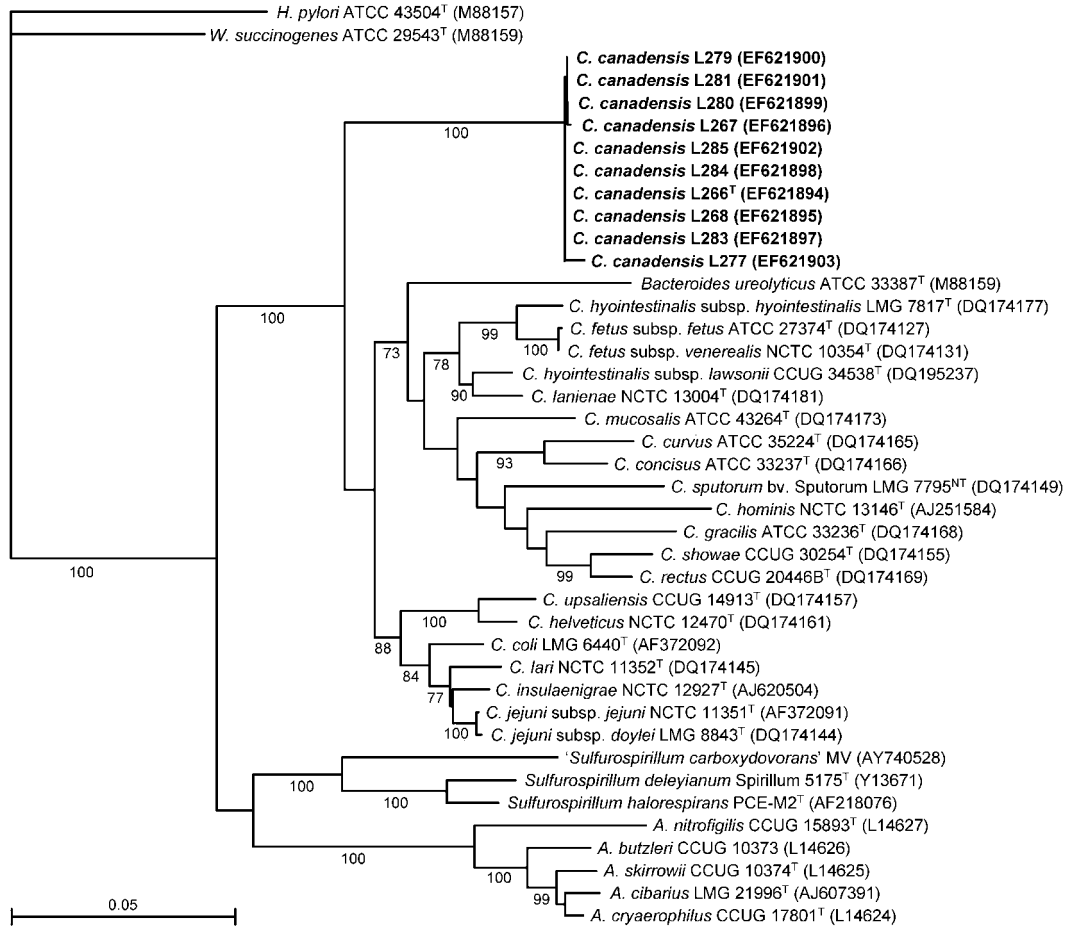

Fig. 3. Rooted tree based on 16S rRNA gene sequences, showing the phylogenetic relationships of ten strains of *C. canadensis* sp. nov. Refer to Fig. 2 for further details.

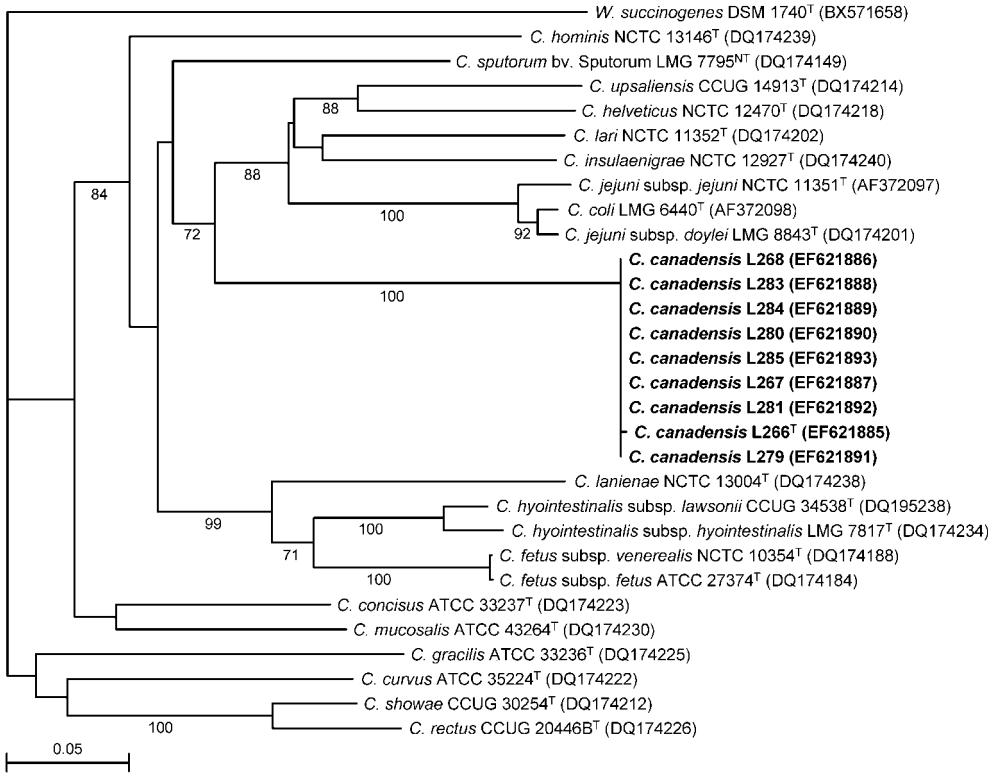

Fig. 4. Rooted tree based on *rpoB* gene sequences, showing the phylogenetic relationships of nine strains of *C. canadensis* sp. nov. Refer to Fig. 2 for further details.

between runs by linking profiles for analysis to their respective molecular mass standard, as described in the software manufacturer's instructions. Normalized AFLP profiles were compared using the Dice coefficient and clustered by the unweighted pair group with mathematical averages (UPGMA) method.

Ten isolates of small, motile, Gram-negative bacilli were isolated from cloacal swabs of captive whooping cranes. All isolates were presumptively identified as *Campylobacter* based on genus-specific PCR, but were not identifiable by *Campylobacter* species-specific PCR. TEM revealed that cells were uniflagellate and pleomorphic, typically sigmoid to allantoid in shape but also spheroid to coccoid (Fig. 1). Sigmoid cells were 2.61 ± 1.72 μm (mean ± SD) in length and 0.48 ± 0.09 μm in width. Spheroid cells were 0.73 ± 0.16 μm in length and 0.59 ± 0.09 μm in width. The following percentages of positive results for physiological characters were observed (n=10): 0 % α-haemolysis, 100 % oxidase, 40 % catalase, 0 % hippurate hydrolysis, 50 % urease, 50 % nitrate reduction, 0 % alkaline phosphatase, 40 % trace H_2S production, 20 % acid production on triple-sugar iron (TSI) agar, 0 % indoxyl acetate hydrolysis, 0 % growth at 25 °C (microaerobic), 100 % growth at 37 °C (microaerobic), 100 % growth at 42 °C (microaerobic), 100 % anaerobic growth at 37 °C, 0 % growth in ambient O_2 at 25 or 37 °C, 0 % growth on nutrient agar, 100 % growth on CCDA, 100 % growth on MacConkey agar, 30 % growth on 1 % glycine, 10 % growth on 3.5 % NaCl, 40 % resistance to nalidixic acid, 0 % resistance to cephalotin, 0 % required H_2 within the microaerobic atmosphere, 80 % γ-glutamyl transpeptidase and 100 % nitrite reduction (Table 1). The bacterium exhibited no or very restricted growth at 30 °C (microaerobically). The most useful characters that distinguished the bacterium from *Arcobacter* species and recognized *Campylobacter* taxa were negative hippurate and indoxyl acetate hydrolysis, production of γ-glutamyl transpeptidase, positive nitrite reduction, inability to grow in ambient 0_2, no growth microaerobically at 25 and 30 °C, inability to grow on nutrient agar and on 3.5 % NaCl, ability to grow anaerobically and on MacConkey agar and sensitivity to cephalotin.

To determine their taxonomic position, the 23S rRNA genes of two isolates (L266^T and L268) were sequenced (approx. 2700 nt). A search of the NCBI database determined that the isolates were most closely related to taxa within the genus *Campylobacter*. Further analysis indicated that the isolates formed a robust clade (100 % bootstrap support) with other *Campylobacter* species, and were distinct from arcobacters (Fig. 2). Phylogenetic relationships were examined further using the 16S rRNA gene (approx. 1400 nt). A search of the NCBI database confirmed that the unidentified isolates were phylogeneti-

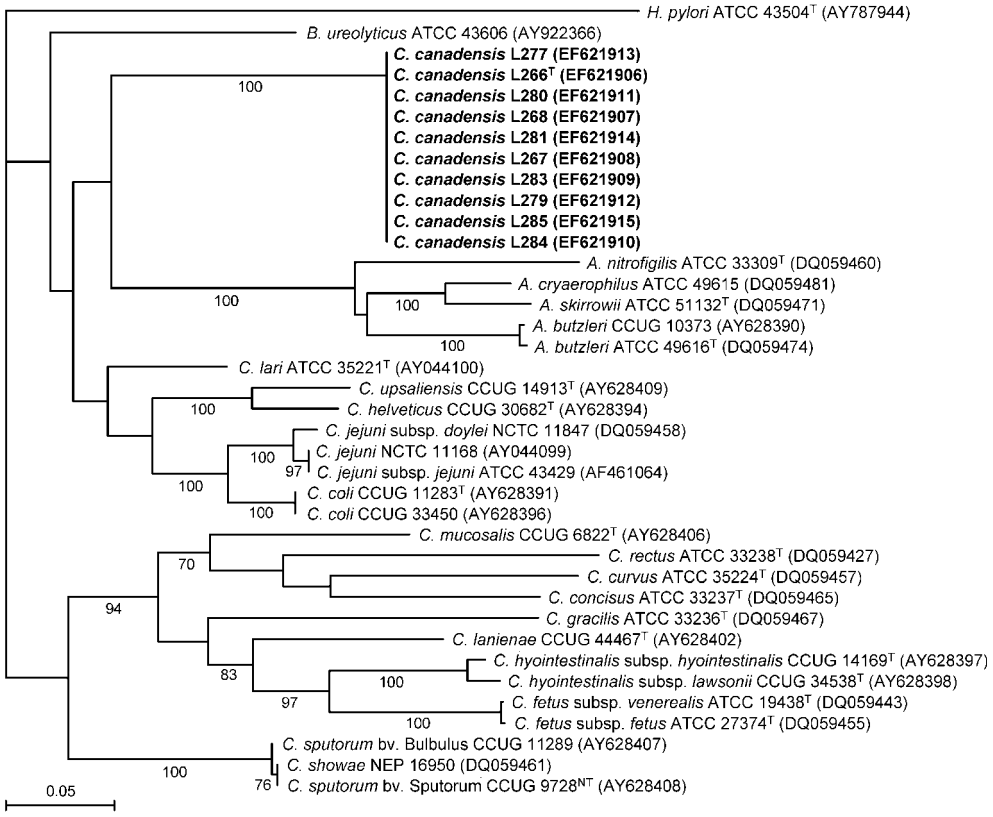

Fig. 5. Rooted tree based on *cpn60* gene sequences, showing the phylogenetic relationships of ten strains of *C. canadensis* sp. nov. Refer to Fig. 2 for further details.

Table 2. DNA relatedness of strains of *C. canadensis* sp. nov. and other members of the genus *Campylobacter*

A ΔT_m greater than 5 °C between the homologous and heterologous hybrids indicates two different species (Mehlen *et al.*, 2004). −, No detectable DNA–DNA hybridization.

Strain	ΔT_m (°C)	
	L266^T	L284
C. canadensis sp. nov. L266^T	0.0	0.0
C. canadensis sp. nov. L284	0.0	0.0
C. coli L7	17.0	−
C. concisus ATCC 33237^T	5.2	−
C. fetus subsp. *fetus* ATCC 25936	−	−
C. hyointestinalis subsp. *hyointestinalis* CCUG 14169^T	−	18.7
C. jejuni ATCC 49943	−	35.7
C. lanienae CCUG 44467^T	11.2	29.1
C. lari NCTC 11352^T	−	−
C. sputorum bv. Sputorum CCUG 9728^T	−	−
C. upsaliensis CCUG 14913^T	−	−

cally most closely related to *Campylobacter*, and pairwise sequence comparisons of L266^T with the type strains of most closely related species revealed similarities of 92 % with *C. jejuni* and *C. coli* and 91 % with *Campylobacter lari*, *C. insulaenigrae*, *C. helveticus* and *C. upsalienesis*. The dissimilarity in sequence with its nearest phylogenetic neighbours was substantially greater than the 3 % divergence typically used to delineate different species based on 16S rRNA gene sequence (Stackebrandt & Ludwig, 1988). The neighbour-joining dendrogram indicated that all ten isolates from whooping cranes formed a robust clade (100 % bootstrap support) which was clearly distinct from *Arcobacter* and *Sulfurospirillum* strains and grouped with *Campylobacter* species (Fig. 3). However, the clade was very distinct from clades containing other *Campylobacter* species.

In view of the low 16S rRNA gene sequence similarity between the unidentified isolates and other *Campylobacter* species, the *rpoB* (approx. 525 nt) and *cpn60* (approx. 450 nt) genes were sequenced and genomic DNA–DNA hybridization was conducted. The *cpn60* and *rpoB* genes have recently been applied to study phylogenetic relationships within the *Campylobacteraceae* (Hill *et al.*, 2006; Kärenlampi *et al.*, 2004; Korczak *et al.*, 2006). Searches

of the NCBI database indicated that the isolates from whooping cranes were most similar to *Campylobacter* species. Pairwise comparisons of sequence similarity between L266^T and *Campylobacter* species were $\leqslant$81 and $\leqslant$93 %, respectively, for the *rpoB* and *cpn60* genes. The unidentified isolates formed robust monophyletic groupings distinct from *Campylobacter* and *Arcobacter* species for both the *rpoB* (Fig. 4) and *cpn60* (Fig. 5) genes. Similarly to Korczak *et al.* (2006), we observed good congruence between *rpoB* and 16S rRNA gene sequence results. In contrast, analysis of the *cpn60* gene indicated that the unidentified isolates were much more closely related to *Arcobacter* species than was indicated by the 23S rRNA, 16S rRNA or *rpoB* gene sequences. The results of DNA–DNA hybridization confirmed that the unidentified isolates are genetically distinct from other *Campylobacter* species (Table 2); a ΔT_m greater than 5.0 °C indicates two different species (Mehlen *et al.*, 2004). Species-specific primers targeting the *cpn60* gene that we developed were found to be specific for the unidentified species. As predicted, an amplicon of approximately 250 nt was observed for all ten isolates recovered from cranes but not for any reference strains of *Campylobacter*, *Arcobacter* or *Helicobacter*.

C. jejuni, *C. coli* and *C. lari* are commonly associated with birds (e.g. Waldenström *et al.*, 2002) and, in addition to the genotypic uniqueness we observed, the isolates from whooping cranes also differed from these three taxa physiologically. Relative to *C. lari*, the crane isolates were unable to grow at 30 °C, grew anaerobically and on MacConkey and nutrient agars, were sensitive to cephalotin and were able to reduce nitrite. The inability to hydrolyse hippurate and indoxyl acetate, the inability to haemolyse blood or grow at 30 °C, the ability to grow anaerobically and on MacConkey and nutrient agars and to reduce nitrite, combined with sensitivity to cephalotin and the ability of some strains to degrade urea, distinguished the whooping crane isolates from *C. jejuni* and *C. coli*. Interestingly, a number of studies have reported unidentified hippurate-negative and urease-positive or urease-negative *Campylobacter* species from a variety of avian sources (e.g. Kaneko *et al.*, 1999; Moore *et al.*, 2002; Waldenström *et al.*, 2002). In some of these studies, it is possible that the *Campylobacter* isolates in question were *C. lari*. However, in others, it is very possible that the unidentified isolates represented the novel taxon described in this study. The primers that we have developed to target the *cpn60* gene should prove useful in this regard.

The results of the polyphasic taxonomic study conducted and described herein clearly indicate that the isolates recovered from captive whooping cranes at the Calgary Zoo represent a unique species of *Campylobacter*, for which we propose the name *Campylobacter canadensis* sp. nov. The epithet was chosen because it pertains to the restricted geography of breeding and nesting of the migratory population of whooping cranes in the wild, and the country in which the birds examined in the current study live. Physiological characters that serve to differentiate *C. canadensis* from *Arcobacter* species as well as other species within the genus *Campylobacter* are presented in Table 1. These characters along with species-specific primers that we developed can be used to identify *C. canadensis*. It is pertinent to note that the ten isolates of *C. canadensis* were recovered from different individuals in 2004 and 2006. AFLP genotyping indicated that considerable genetic variability existed within the strains examined (Fig. 6). Interestingly, two isolates (L267 and L281) were determined to be identical but were isolated 2 years apart and from different individuals.

Description of *Campylobacter canadensis* sp. nov.

Campylobacter canadensis (ca.na.den′sis. N.L. masc. adj. *canadensis* pertaining to Canada).

Cells are Gram-negative, motile, polymorphic in shape (sigmoid to coccoid), 0.5–0.6 μm wide and 0.7–2.6 μm

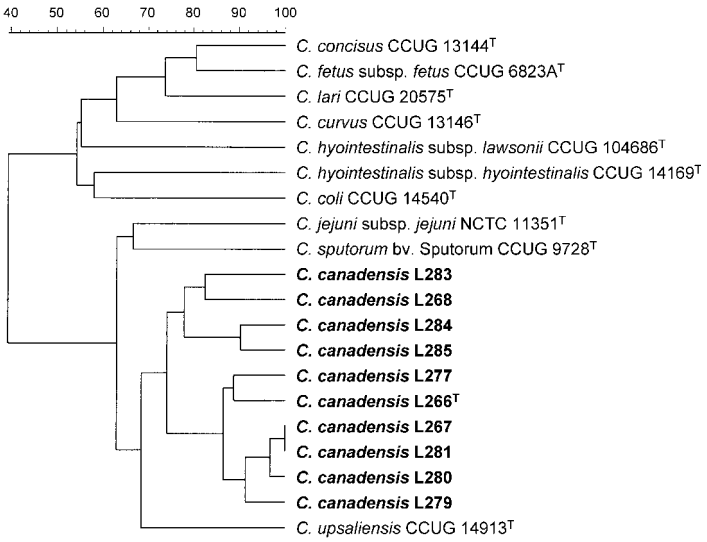

Fig. 6. Dendrogram of AFLP genotypes.

long, possessing a single polar flagellum. On Karmali agar, colonies are whitish-grey in colour with a light centre, margins are entire and the colony surfaces are typically smooth and flat. Colonies are 0.3–2.0 mm in diameter at 37 and 40 °C after 48 h. Colony appearance on CCDA, Columbia agar and trypticase soy agar is similar to that on Karmali agar, but growth on these media is slightly restricted relative to Karmali agar. Growth on Mueller–Hinton agar is very restricted. The bacterium is not α-haemolytic. All strains are microaerophilic and thermophilic, able to grow well at 40 and 42 °C. Under microaerobic conditions, no isolates grow at 25 °C and no or highly restricted growth occurs at 30 °C; all isolates grow at 35 and 37 °C. All isolates also grow under anaerobic conditions. No detectable growth occurs under aerobic conditions at 37 or 40 °C. None of the isolates hydrolyse indoxyl acetate or hippurate, produce alkaline phosphatase or require H_2 to grow. All isolates produce oxidase, grow on MacConkey agar and reduce nitrite. No isolates grow on nutrient agar. Some isolates reduce nitrate (50 %), produce catalase (40 %), urease (50 %) and γ-glutamyl transpeptidase (80 %), grow on 1 % glycine (30 %) and 3.5 % NaCl (10 %), are resistant to nalidixic acid (40 %) and produce acid (20 %) or H_2S (40 %) in TSI agar. All isolates are susceptible to cephalotin. No growth occurs in the presence of 2.0 or 3.5 % (w/v) NaCl at 40 °C.

The type strain is L266^T (=CCUG 54429^T =LMG 24001^T). This strain and other known strains were isolated from the cloacae of captive whooping cranes at the Calgary Zoo in 2004 and 2006.

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References

Felsenstein, J. (2005). PHYLIP (phylogeny inference package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.

Foster, G., Holmes, B., Steigerwalt, A. G., Lawson, P. A., Thorne, P., Byrer, D. E., Ross, H. M., Xerry, J., Thompson, P. M. & Collins, M. D. (2004). *Campylobacter insulaenigrae* sp. nov., isolated from marine mammals. *Int J Syst Evol Microbiol* **54**, 2369–2373.

Han, Y. H., Smibert, R. M. & Krieg, H. R. (1991). *Wolinella recta*, *Wolinella curva*, *Bacteroides ureolyticus*, and *Bacteroides gracilis* are microaerophiles, not anaerobes. *Int J Syst Bacteriol* **41**, 218–222.

Hill, J. E., Paccagnella, A., Law, K., Melito, P. L., Woodward, D. L., Price, L., Leung, A. H., Ng, L.-K., Hemmingsen, S. M. & Goh, S. H. (2006). Identification of *Campylobacter* spp. and discrimination from *Helicobacter* and *Arcobacter* spp. by direct sequencing of PCR-amplified *cpn60* sequences and comparison to cpnDB, a chaperonin reference sequence database. *J Med Microbiol* **55**, 393–399.

Hoar, B. M., Whiteside, D. P., Ward, L., Inglis, G. D. & Morck, D. W. (2007). Evaluation of the enteric microflora of captive whooping cranes (*Grus americana*) and sandhill cranes (*Grus canadensis*). *Zoo Biol* **26**, 141–153.

Inglis, G. D. & Kalischuk, L. D. (2003). Use of PCR for direct detection of *Campylobacter* species in bovine feces. *Appl Environ Microbiol* **69**, 3435–3447.

Inglis, G. D., McConville, M. & de Jong, A. (2006). Atypical *Helicobacter canadensis* strains associated with swine. *Appl Environ Microbiol* **72**, 4464–4471.

Kaneko, A., Matsuda, M., Miyajima, M., Moore, J. E. & Murphy, P. G. (1999). Urease-positive thermophilic strains of *Campylobacter* isolated from seagulls (*Larus* spp.). *Lett Appl Microbiol* **29**, 7–9.

Kärenlampi, R. I., Tolvanen, T. P. & Hänninen, M.-L. (2004). Phylogenetic analysis and PCR-restriction fragment length polymorphism identification of *Campylobacter* species based on partial *groEL* gene sequences. *J Clin Microbiol* **42**, 5731–5738.

Kokotovic, B. & On, S. L. (1999). High-resolution genomic fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* by analysis of amplified fragment length polymorphisms. *FEMS Microbiol Lett* **173**, 77–84.

Korczak, B. M., Stieber, R., Emler, S., Burnens, A. P., Frey, J. & Kuhnert, P. (2006). Genetic relatedness within the genus *Campylobacter* inferred from *rpoB* sequences. *Int J Syst Evol Microbiol* **56**, 937–945.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

Lawson, A. J., On, S. L. W., Logan, J. M. J. & Stanley, J. (2001). *Campylobacter hominis* sp. nov., from the human gastrointestinal tract. *Int J Syst Evol Microbiol* **51**, 651–660.

Logan, J. M. J., Burens, A., Linton, D., Lawson, A. J. & Stanley, J. (2000). *Campylobacter lanienae* sp. nov., a new species isolated from workers in an abattoir. *Int J Syst Evol Microbiol* **50**, 865–872.

Mehlen, A., Goeldner, M., Ried, S., Stindl, S., Ludwig, W. & Schleifer, K. H. (2004). Development of a fast DNA-DNA hybridization method based on melting profiles in microplates. *Syst Appl Microbiol* **27**, 689–695.

Moore, J. E., Gilpin, D., Crothers, E., Canney, A., Kaneko, A. & Matsuda, M. (2002). Occurrence of *Campylobacter* spp. and *Cryptosporidium* spp. in seagulls (*Larus* spp.). *Vector Borne Zoonotic Dis* **2**, 111–114.

Nicholas, K. B. & Nicholas, H. B. (1997). GeneDoc: a tool for editing and annotating multiple sequence alignments. http://www.nrbsc.org/gfx/genedoc/index.html

On, S. L. W., Holmes, B. & Sackin, M. J. (1996). A probability matrix for the identification of campylobacters, helicobacters and allied taxa. *J Appl Bacteriol* **81**, 425–432.

Page, R. D. M. (2001). TreeView. http://taxonomy.zoology.gla.ac.uk/rod/rod.html

Sallen, B., Rajoharison, A., Desvarenne, S., Quinn, F. & Mabilat, C. (1996). Comparative analysis of 16S and 23S rRNA sequences of *Listeria* species. *Int J Syst Bacteriol* **46**, 669–674.

Stackebrandt, E. & Ludwig, W. (1988). 16S ribosomal RNA cataloging. *Methods Enzymol* **167**, 132–138.

Vandamme, P., Falsen, E., Rossau, R., Hoste, B., Segers, P., Tytgat, R. & De Ley, J. (1991). Revision of *Campylobacter*, *Helicobacter*, and *Wolinella* taxonomy: emendation of generic descriptions and proposal of *Arcobacter* gen. nov. *Int J Syst Bacteriol* **41**, 88–103.

Waldenström, J., Broman, T., Carlsson, I., Hasselquist, D., Achterberg, R. P., Wagenaar, J. A. & Olsen, B. (2002). Prevalence of *Campylobacter jejuni*, *Campylobacter lari*, and *Campylobacter coli* in different ecological guilds and taxa of migrating birds. *Appl Environ Microbiol* **68**, 5911–5917.