

## Species of the Family *Helicobacteraceae* Detected in an Australian Sea Lion (*Neophoca cinerea*) with Chronic Gastritis

Andrew P. A. Oxley,<sup>1,3\*</sup> Mark Powell,<sup>2</sup> and David B. McKay<sup>3</sup>

School of Biological, Cellular, and Molecular Sciences, University of New England, Armidale, New South Wales 2351,<sup>1</sup>  
Noosa Veterinary Surgery, Tewantin, Queensland 4565,<sup>2</sup> and Faculty of Science, University of  
the Sunshine Coast, Maroochydore District Capital, Queensland 4558,<sup>3</sup> Australia

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**We describe the first case of gastritis in a male Australian sea lion (*Neophoca cinerea*) in which members of the family *Helicobacteraceae*, particularly the genus *Wolinella*, were detected. The sea lion exhibited clinical signs of gastrointestinal disease, including abdominal pain, lack of appetite, and lethargy. Examination of one ileal and five gastric biopsy specimens collected over a 10-year period revealed persistent fibrosis and/or superficial focal erosion and ulceration of the lamina propria. Spiral-shaped organisms 5 to 12  $\mu\text{m}$  long were observed in two of the gut biopsy specimens. While *Helicobacter* species were detected by PCR in one of the gastric biopsy specimens, *Wolinella* species were detected in four of the five gastric specimens, including those in which spiral-shaped organisms were observed. Comparisons of biopsy specimen ribosomal DNA sequences with those obtained from the feces of this animal, the gastric tissue of a clinically healthy individual, and the feces of several other cohoused sea lions and fur seals revealed a separate and possibly novel gastric *Helicobacter* species. A possibly novel *Wolinella* species, along with *Wolinella succinogenes*, was also identified. These findings highlight the pathogenic potential of other members of this family in the etiopathogenesis of gastric disease in these animals.**

Members of the family *Helicobacteraceae*, particularly the *Helicobacter* species, have been recognized as agents of gastrointestinal disease in both humans and a broad range of animal hosts (11, 26). While not all species are pathogenic (25), colonization of the gut by such organisms (particularly *Helicobacter pylori*) may lead to conditions of gastroenteritis, gastric ulceration, and gastric adenocarcinoma (1, 9). The genus *Wolinella*, like the genus *Helicobacter*, belongs to the epsilon subclass of the proteobacteria and is a member of the family *Helicobacteraceae* (29). However, as a natural inhabitant of the rumen of cattle (31), its role as a pathogen has not been described. In this article, we describe a case of chronic gastritis in a male Australian sea lion (*Neophoca cinerea*) and, through an examination of other, asymptomatic cohoused fur seals and sea lions, discuss the potential role of the family *Helicobacteraceae* in the etiopathogenesis of gastric disease in these animals.

### CASE REPORT

In early March 1992, a 4-year-old male Australian sea lion (*N. cinerea*) that was named Duran and that had abdominal pain, lack of appetite, and lethargy was examined for a possible obstruction of the gastrointestinal tract. However, an exploratory laparotomy revealed no apparent obstruction. Instead, gas-filled loops of the small intestine that were purple in color and that had obvious poor perfusion were observed. A biopsy of the ileum revealed eosinophilic enteritis that was tentatively attributed to an enteric allergenic response. While the nature of the allergen was unclear, it was proposed that parasitic antigens and/or food allergens might cause similar reactions. How-

ever, despite the absence of any obvious etiological agent, the animal was subsequently treated for *Helicobacter* species infection over a 2-week period with amoxicillin (10 mg/kg of body weight twice daily) (SmithKline Beecham Pharmaceuticals, Dandenong, Victoria, Australia), prednisolone (30 mg daily) (Mavlab Pty Ltd., Slacks Creek, Queensland, Australia), and metronidazole (10 mg/kg twice daily) (Alphapharm Pty Ltd., Glebe, New South Wales, Australia).

While the animal exhibited some clinical signs of recovery after several days of treatment (bright, alert, and with an increased appetite), the animal appeared to relapse 2 weeks later. Further biopsy specimens obtained endoscopically from the stomach of the animal (in late March 1992) revealed the presence of active ulcerative gastritis in conjunction with some poorly preserved spiral-shaped structures suggestive of *Helicobacter*. The animal was subsequently continued on the previous antibiotic treatment regimen.

A substantial improvement in the animal's condition was reported several weeks later (mid-April 1992) and was substantiated by resolving gastritis noted in a second endoscopic examination of the stomach. Nevertheless, the animal continued to exhibit intermittent signs of abdominal discomfort in association with a loss of appetite and a strange hiccough-like behavior between May 1992 and March 2002. Broad-spectrum antibiotics (amoxicillin at 10 mg/kg twice daily [SmithKline Beecham] and omeprazole at 20 mg daily [Astrazeneca Pty Ltd., North Ryde, New South Wales, Australia]) were periodically continued, and further biopsy specimens were taken from the stomach in May 1992, May 2000, and March 2002 for histopathological evaluation.

### MATERIALS AND METHODS

**Histopathological analysis.** One ileal and five gastric biopsy specimens were obtained from a male Australian sea lion (*N. cinerea*) named Duran between 1992 and 2002 (A1 to A6). Also, as a control, a gastric biopsy specimen was

\* Corresponding author. Mailing address: Faculty of Science, University of the Sunshine Coast, Maroochydore District Capital, Queensland 4558, Australia. Phone: 61 7 5459 4449. Fax: 61 7 5430 1276. E-mail: aoxley@usc.edu.au.

obtained from a clinically healthy and cohoused female Australian sea lion named Portia in 1992. Tissue samples were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at a thickness of 2 to 4  $\mu\text{m}$ . Samples were subsequently stained with hematoxylin and eosin (HE) and examined microscopically for signs of infection (inflammation and/or ulceration) like those associated with *Helicobacter* infections in other animals.

**Collection of fecal samples.** Fecal samples were collected in 2003 from the nightly enclosures of Duran, Portia, and four other cohoused fur seals and sea lions (Bella, Groucher, Massey, and Shilo) from UnderWater World (Mooloolaba, Queensland, Australia). A 20-g sample was collected from each individual, placed in a sterile collection vial containing 40 ml of sterile phosphate-buffered saline supplemented with 20% glycerol, mixed, and placed immediately on ice.

**Total DNA extraction.** For each biopsy specimen, approximately 15 mg of paraffin-embedded tissue was removed with a sterile scalpel blade and added to a sterile 2-ml microcentrifuge tube. Total DNA was extracted from the sections by using a QIAamp DNA minikit (Qiagen, Clifton Hill, Victoria, Australia) and reloaded in 100  $\mu\text{l}$  of elution buffer (10 mM Tris-Cl, pH 8.5).

Fecal samples were homogenized for 2 min, and a 200- $\mu\text{l}$  aliquot (or approximately 200 mg) was removed and placed in a sterile 2-ml microcentrifuge tube. Total DNA was extracted from the feces by using a QIAamp DNA minikit (Qiagen) and reloaded in 200  $\mu\text{l}$  of elution buffer (10 mM Tris-Cl, pH 8.5).

**PCR conditions.** To establish whether *Helicobacter* species were present in biopsy specimens or fecal samples, the following genus-specific primers were used to produce 16S rRNA amplicons approximately 400 bp long: 5'-TATGACGGGTATCCGGC-3' (H276 forward) and 5'-ATTCCACCTACTCTCCCA-3' (H676 reverse) (12). Total volumes of 30  $\mu\text{l}$  were used in all PCRs, which were performed by using a Mastercycler gradient (Eppendorf, Hamburg, Germany). Reaction mixtures contained 25 pmol of each primer, 1 $\times$  PCR buffer [67 mM Tris-HCl (pH 8.8), 16.6 mM  $(\text{NH}_4)_2\text{SO}_4$ , 0.45% Triton X-100, 0.2 mg of gelatin/ml], 2.5 mM  $\text{MgCl}_2$ , 170  $\mu\text{M}$  deoxyribonucleoside triphosphate, 1.0 U of *Taq* polymerase (Biotech International, Perth, Western Australia, Australia), and 2  $\mu\text{l}$  of DNA extract. All reactions were heated at 94°C for 2 min and then subjected to 30 cycles consisting of denaturation at 94°C for 30 s, annealing at 66°C for 30 s, and extension at 72°C for 30 s. For biopsy specimens, a 2- $\mu\text{l}$  aliquot from each of the initial PCRs (including the controls [see below]) was added to the above reaction mixture and subjected to a second round of amplification (total of 60 cycles). A 5- $\mu\text{l}$  aliquot from each of the PCRs was separated by electrophoresis in a 1% agarose gel, stained with ethidium bromide, and visualized under UV light. The image was recorded by using GeneSnap (version 4.00.00) (Syngene [a division of Synoptics Ltd.], Cambridge, England).

**PCR controls.** To verify the results of the PCRs, several controls were included with each series of reactions. These included one positive and two negative controls. The positive control was used to verify the success of the PCR and contained 2  $\mu\text{l}$  of *H. pylori* strain 26695 genomic DNA (School of Microbiology and Immunology, University of New South Wales, Sydney, New South Wales, Australia). The negative controls were used to detect the presence of false amplification products and contained either 2  $\mu\text{l}$  of *Escherichia coli* genomic DNA or 2  $\mu\text{l}$  of water.

**Cloning, plasmid isolation, and sequencing.** Amplicons were cloned by using a pGEM-T Easy Vector system (Promega). In accordance with the manufacturer's instructions, 3  $\mu\text{l}$  of PCR product was ligated with 50 ng of vector at room temperature for 1 h and transformed with high-efficiency JM109 competent cells (Promega). Clones were selected by using ampicillin plates containing isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside (X-Gal) and screened for inserts by using a CloneChecker system (Invitrogen, Mulgrave, Queensland, Australia). Plasmid DNA was isolated from *E. coli* JM109 cells by using a Wizard Plus SV Minipreps DNA purification system (Promega). DNA was submitted to the Australian Genome Research Facility and sequenced with an ABI Prism BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif.).

**Sequence analysis.** Sequences obtained from both biopsy and fecal samples were entered into the BioEdit sequence alignment editor for Windows 95/98/NT (version 5.0.9) (15) and aligned by using CLUSTAL W (version 1.4) (28). Sequences retrieved from the GenBank database for *H. pylori*, *Arcobacter nitrofiglis*, *Sulfurospirillum deleyianum*, and several *Campylobacter* and *Wolinella* species were included with this alignment as reference sequences. Also, sequences from both biopsy and fecal samples were aligned with sequences of other *Helicobacter* and *Campylobacter* species retrieved from GenBank to determine the variation (if any) between these sequences and those of other species. A difference in nucleotide sequence of approximately 3% or greater between any two ribosomal DNAs was used to distinguish a separate species (5, 10, 18, 27). Tree diagrams representing these alignments were constructed with the Molecular

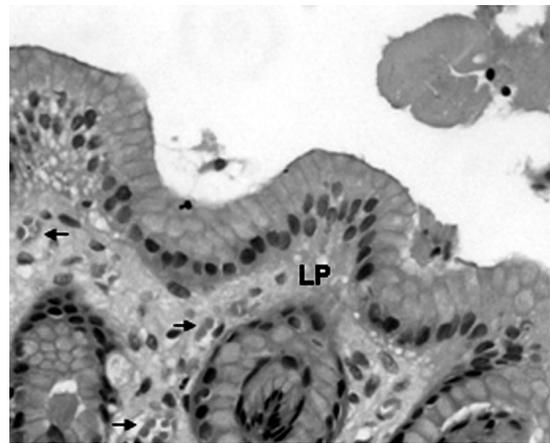


FIG. 1. Histological section B211685 (control) from the stomach of Portia, a clinically healthy female Australian sea lion (*N. cinerea*). Several small focal but loose accumulations of lymphocytes and plasma cells (arrows) are observed within the lamina propria (LP). No fibrosis, ulceration, or spiral-shaped bodies are evident. Hematoxylin and eosin stain; magnification:  $\times 340$ .

Evolutionary Genetics Analysis program (version 2.1) (19) by using the neighbor-joining method.

**Nucleotide sequence accession numbers.** Sequences determined in this study were deposited under the following GenBank accession numbers: for Duran's biopsy specimens—B210210 (A2), AY501370; B211689 (A3), AY501371; B214747 (A4), AY501372; B019694 (A5), AY501373; and B212633 (A6), AY501374; for Portia's biopsy specimen—B211685, AY606046; and for the feces of the captive seals—Duran (DN210503), AY501375; Portia (PT210503), AY501378; Bella (BE210503), AY501376; Shilo (SH210503), AY501377; Groucher (GH210503), AY501399; and Massey (MY210503), AY501340.

## RESULTS

**Histopathological findings.** One ileal and five gastric biopsy specimens taken from Duran over a 10-year period as well as a control specimen taken from a clinically healthy female sea lion (Portia) were examined for pathological features consistent with infections caused by members of the genus *Helicobacter*. Photomicrographs of the control specimen and several gastric biopsy specimens exhibiting distinct pathological features and/or the presence of a possible etiological agent from Duran are shown in Fig. 1 to 3.

Small focal but inconsequential loose accumulations of lymphocytes and plasma cells were detected in the lamina propria of the control gastric tissue specimen collected from Portia (Fig. 1). As these changes occurred in the absence of fibrosis, ulceration, and spiral-shaped bodies, they may be considered to be within normal limits and thus may be representative of the normal gastric mucosa.

Mild to moderate enteritis characterized by a hypercellular lamina propria consisting of infiltrates of eosinophils, mast cells, lymphocytes, and plasma cells was observed for the ileal biopsy specimen (A1) obtained in March 1992 (data not shown). While these features may be indicative of an enteric allergic response, no etiological agent was observed. For the gastric biopsy specimen (A2) collected in April 1992, however, some spiral-shaped bodies approximately 10  $\mu\text{m}$  in length (although poorly preserved) were observed in the superficial mucus layer (Fig. 2). In comparison with the findings for the

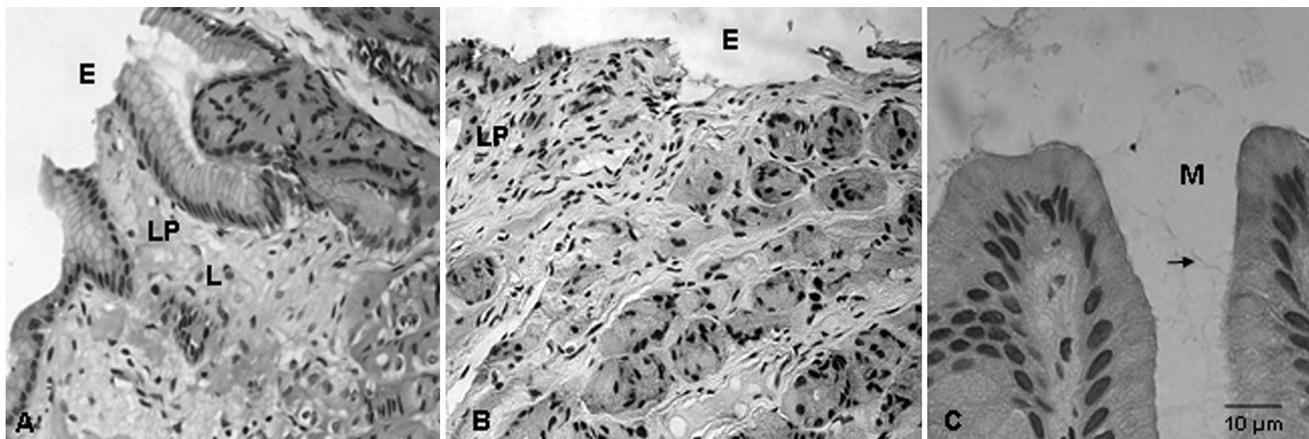


FIG. 2. Histological section B210210 (A2) from the stomach of Duran, a male Australian sea lion with gastritis. (A and B) The gastritis is characterized by fibrosing lymphocytic-plasmacytic (L) infiltration of the lamina propria (LP), with focal superficial erosion and ulceration (E). (C) Small numbers of poorly preserved spiral-shaped bodies (arrow) are observed in the superficial mucus layer (M). HE stain; magnifications: A and B,  $\times 400$ ; C,  $\times 1,000$ .

control (Fig. 1), these spiral-shaped bodies occurred in association with moderate to severe gastritis, which was characterized by superficial focal erosion, ulceration, and fibrosis of the lamina propria with infiltrates of lymphocytes; these features most likely represented the etiological agent. However, no spiral-shaped bodies were observed in a second gastric biopsy specimen (A3) taken approximately 14 days later (data not shown). With a focal loss of glands and replacement fibrosis associated with a mild infiltration of monocytes in the lamina propria, this specimen was in marked contrast to specimen A2 and thus may have indicated resolving gastritis. Nevertheless, mild gastritis featuring fibrosing lymphocytic infiltration of the lamina propria and congestion of the superficial mucosa was observed for the gastric biopsy specimen (A4) obtained 4 weeks later, in May 1992 (data not shown). However, no spiral-shaped bodies were observed in this specimen.

For the gastric biopsy specimen (A5) collected 10 years later, in May 2000, an expanded lamina propria featuring congestion, focal hemorrhage, and infiltration of lymphoid cells and neutrophils was observed (Fig. 3). In contrast to the findings for the control specimen and Duran's previous biopsy

specimens, immense numbers of spiral-shaped organisms approximately 5 to 12  $\mu\text{m}$  in length were observed in the superficial mucus layer.

However, no spiral-shaped organisms were observed in the gastric biopsy specimen (A6) taken in March 2002. Despite this finding, mild gastritis characterized by fibrosis of the superficial submucosa with infiltrates of mononuclear cells was apparent (data not shown).

**PCR detection of species of the family *Helicobacteraceae*.** PCR analysis of paraffin-embedded gut biopsy specimens collected from Duran over a 10-year period is shown in Fig. 4. Amplicons of the expected size of approximately 400 bp were observed for all gastric biopsy specimens collected between 1992 and 2002 (A2 to A6). However, no amplification product was observed for the ileal biopsy specimen collected in March 1992 (A1). Interestingly, while a product was observed for biopsy specimen A5, which featured immense numbers of spiral-shaped organisms, products also were observed for biopsy specimens in which no such organisms were seen (A3, A4, and A6).

Amplicons of the expected size of approximately 400 bp

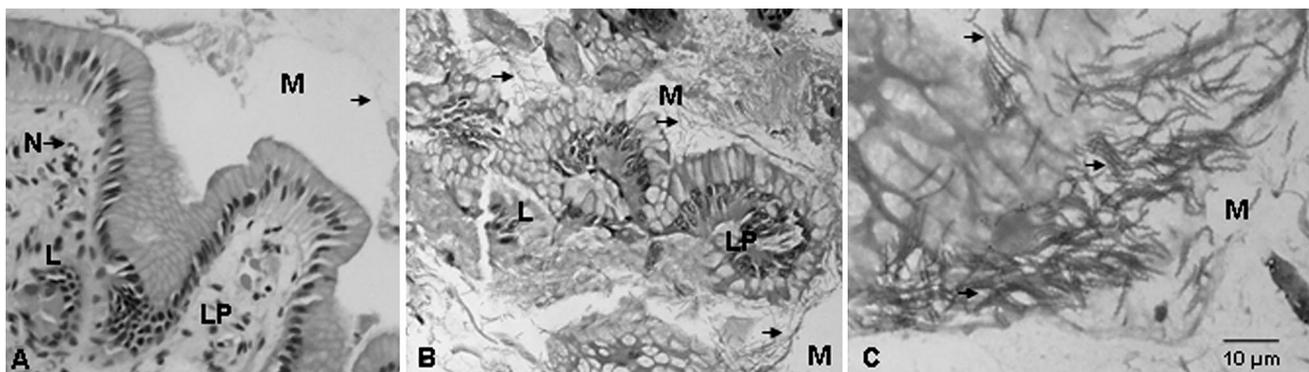


FIG. 3. Histological section B019694 (A5) from the stomach of Duran, a male Australian sea lion with gastritis. (A to C) The lamina propria (LP) is expanded and features congestion and infiltrates of lymphoid cells (L) and neutrophils (N). Immense numbers of spiral-shaped bodies (arrows) are observed in the superficial mucus layer (M). HE stain; magnifications: A and B,  $\times 400$ ; C,  $\times 1,000$ .

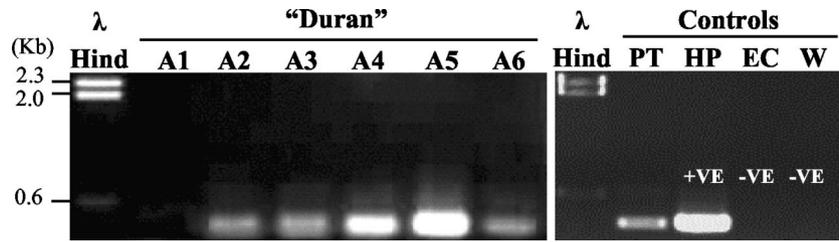


FIG. 4. Agarose gel electrophoresis of PCR products produced from total DNA extracted from paraffin-embedded gut biopsy specimens from Duran, a male Australian sea lion. A1 to A6 correspond to the code numbers used in the histological images. Experimental controls include the following: PT, total DNA extract from a paraffin-embedded control biopsy specimen (B211685) taken from a clinically healthy sea lion named Portia; HP, *H. pylori* genomic DNA; EC, *E. coli* genomic DNA; W, water (no template); λ Hind, molecular weight marker (λ phage DNA cut with HindIII).

were also observed for the control biopsy specimen taken from Portia in 1992 (Fig. 4) and the fecal samples collected from Duran, Portia, and the other cohoused seals in 2003 (data not shown).

**Sequence analysis.** A tree demonstrating the alignment of 16S rRNA sequences from amplicons recovered from gastric biopsy specimens collected from Duran over a 10-year period (1992 to 2002), the gastric biopsy specimen collected from

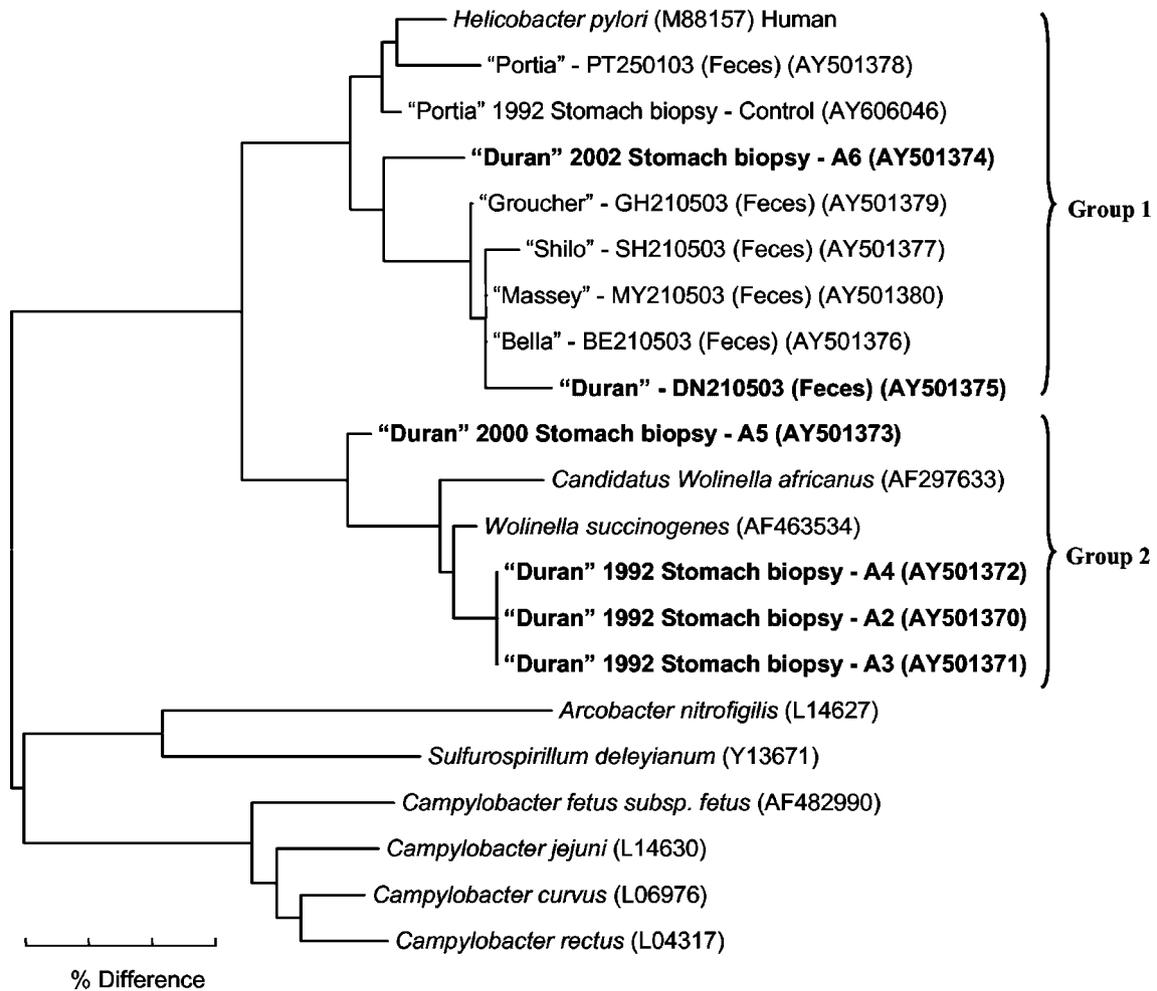


FIG. 5. Neighbor-joining tree demonstrating the 16S rRNA sequence identity among species of the family *Helicobacteraceae* in gut biopsy specimens collected from a male Australian sea lion (Duran) over several years, a gut biopsy specimen collected from a clinically healthy sea lion (Portia), and fecal material collected from Duran, Portia, and several other cohoused seals. Code numbers for biopsy samples are included and correspond to those used in the histological images. *H. pylori*, *Wolinella* spp., and *Campylobacter* spp. are included as reference sequences. The scale bar represents a 3% difference in nucleotide sequences. GenBank accession numbers are given in parentheses. Specimens with sequences of interest are shown in boldface characters.

Portia in 1992, and fecal samples obtained from Duran, Portia, and several other cohoused seals in 2003 is shown in Fig. 5. Two main groups of sequences differing by more than 20 bp (95% identity) were observed. Group 1 consisted of sequences that clustered with the *Helicobacter* type species *H. pylori* and included both a fecal sequence and a gastric biopsy sequence (A6) obtained from Duran in 2003 and 2002, respectively. Sequences obtained from the gut biopsy and fecal specimens from Portia as well as fecal specimens from the other cohoused seals (Shilo, Massey, Groucher, and Bella) also clustered within this group. While a difference of no more than 6 bp (98.4% identity) was observed between sequences obtained from fecal specimens from Duran and the other seals (with the exception of Portia), a difference of 11 to 16 bp (96 to 97.3% identity) was observed between these sequences and the sequence obtained from gastric biopsy specimen A6 from Duran. The sequence obtained from gastric biopsy specimen A6 from Duran thus may represent a *Helicobacter* species distinct from that obtained from his feces or the feces from the other seals.

While the sequence obtained from Portia's gastric biopsy specimen differed from that obtained from her feces by no more than 7 bp (98% identity), a difference of 10 to 16 bp (96 to 97.4% identity) was observed among this sequence, the sequence obtained from gut biopsy specimen A6 from Duran, and the sequences obtained from the feces from all of the other seals. The sequence obtained from Portia's gastric tissue thus may represent a *Helicobacter* species distinct from that obtained from gut biopsy specimen A6 from Duran or the feces from the other seals.

Interestingly, group 2 consisted of sequences from the *Wolinella* type species *Wolinella succinogenes*, a sequence from a putative *Wolinella* species ("*Candidatus Wolinella africanus*"), and sequences obtained from four gastric biopsy specimens collected from Duran in 1992 and 2000 (A2, A3, A4, and A5). While this finding may demonstrate the apparent lack of *Helicobacter* specificity in the current PCR assay, it is surprising that given the large numbers of spiral-shaped organisms observed in biopsy specimen A5, a sequence similar to that of the genus *Wolinella* was recovered. No difference in nucleotide sequence (100% identity) was observed among sequences obtained from biopsy specimens A2, A3, and A4; these sequences thus may represent the same species. Moreover, no more than a 5-bp difference (98.7% identity) was observed between these sequences and the *W. succinogenes* sequence; these sequences thus may represent this organism. The sequence obtained from gastric biopsy specimen A5, however, differed from these gastric biopsy specimen sequences and the *Wolinella* sp. sequence by 11 to 14 bp (96.5 to 97.2% identity); this sequence thus may represent a separate and possibly novel *Wolinella* species.

A tree diagram representing the alignment of 16S rRNA sequences obtained from the fecal and gastric biopsy specimens collected from Duran and Portia, the fecal specimens collected from the other cohoused seals, and other bacterial species contained in the GenBank database is shown in Fig. 6. Despite a comparison with 40 other *Helicobacter* species, sequences obtained from four of the five gastric biopsy specimens collected from Duran (A2, A3, A4, and A5) remained as a cluster with members of the genus *Wolinella*. Only the sequence obtained from one biopsy specimen (A6) clustered with members of the genus *Helicobacter*. In particular, this

sequence, although clustering with a *Helicobacter* species previously identified from pigs (namely, *Helicobacter suis*), differed from this sequence and all other sequences by at least 12 bp (97% identity) and thus may represent a novel species.

Interestingly, while the sequence obtained from Duran's fecal specimens differed from those obtained from his biopsy specimens, this sequence (with a difference of 4 to 11 bp [97.2 to 98.9% identity]) clustered with *Helicobacter* species previously identified from other marine mammals, including harp seals (*Helicobacter* sp. strain MIT 01-5529A), sea lions (*Helicobacter* sp. strain MIT 02-5519C), and sea otters (*Helicobacter* sp. strain MIT 01-5924). In addition, with the exception of Portia (whose sequences clustered with *Helicobacter* species from dolphins and whales), sequences obtained from the feces of all of the other seals, differing by no more than 0 to 8 bp (97.9 to 100% identity), also clustered with this group of sequences.

## DISCUSSION

In this article, we report on the occurrence of species of the family *Helicobacteraceae* within the gut of a male Australian sea lion with gastritis. Despite continued treatment, this condition was (in comparison to the control) characterized by persistent inflammation and/or ulceration of the superficial gastric mucosa over a 10-year period (March 1992 to March 2002). While no etiological agent was observed for the majority of biopsy specimens, spiral-shaped organisms approximately 5 to 12  $\mu\text{m}$  in length were apparent in two gastric biopsy specimens exhibiting marked erosion, ulceration, and fibrosis of the lamina propria (A2 and A5). Interestingly, given that such organisms appear to be morphologically consistent with members of the genus *Helicobacter* that cause similar conditions of disease in humans and a broad range of animal hosts (10, 26), such organisms may represent the etiological agent in this animal. However, considering that infections by such organisms may often be persistent despite treatment (23), the inability of the current antibiotic treatment regimen (10 mg of amoxicillin/kg twice daily) to effectively treat this animal requires review.

Sequences similar to those of the genus *Wolinella* rather than to those of the genus *Helicobacter* were, however, obtained from PCR products from four of the six biopsy specimens collected from Duran, including specimens in which spiral-shaped organisms were observed (A2 and A5). Interestingly, for three of these specimens (A2, A3, and A4), a sequence similar to the sequence of the type species *W. succinogenes* was recovered. This species, despite the absence of evidence to support it being pathogenic in humans or animals, supports numerous virulence genes homologous to those in other closely related and well-recognized pathogenic species (namely, *H. pylori* and *Campylobacter jejuni*) (2). Given that this species maintains curved or helical rod-shaped features (20) similar to those observed for many *Helicobacter* species (26), it is possible that this species represents the spiral-shaped organisms observed in these specimens. Nevertheless, its occurrence in this host is unique and reflects an ecological niche outside the bovine rumen (2). Moreover, its detection in association with gastritis has not been previously reported and merits further examination.

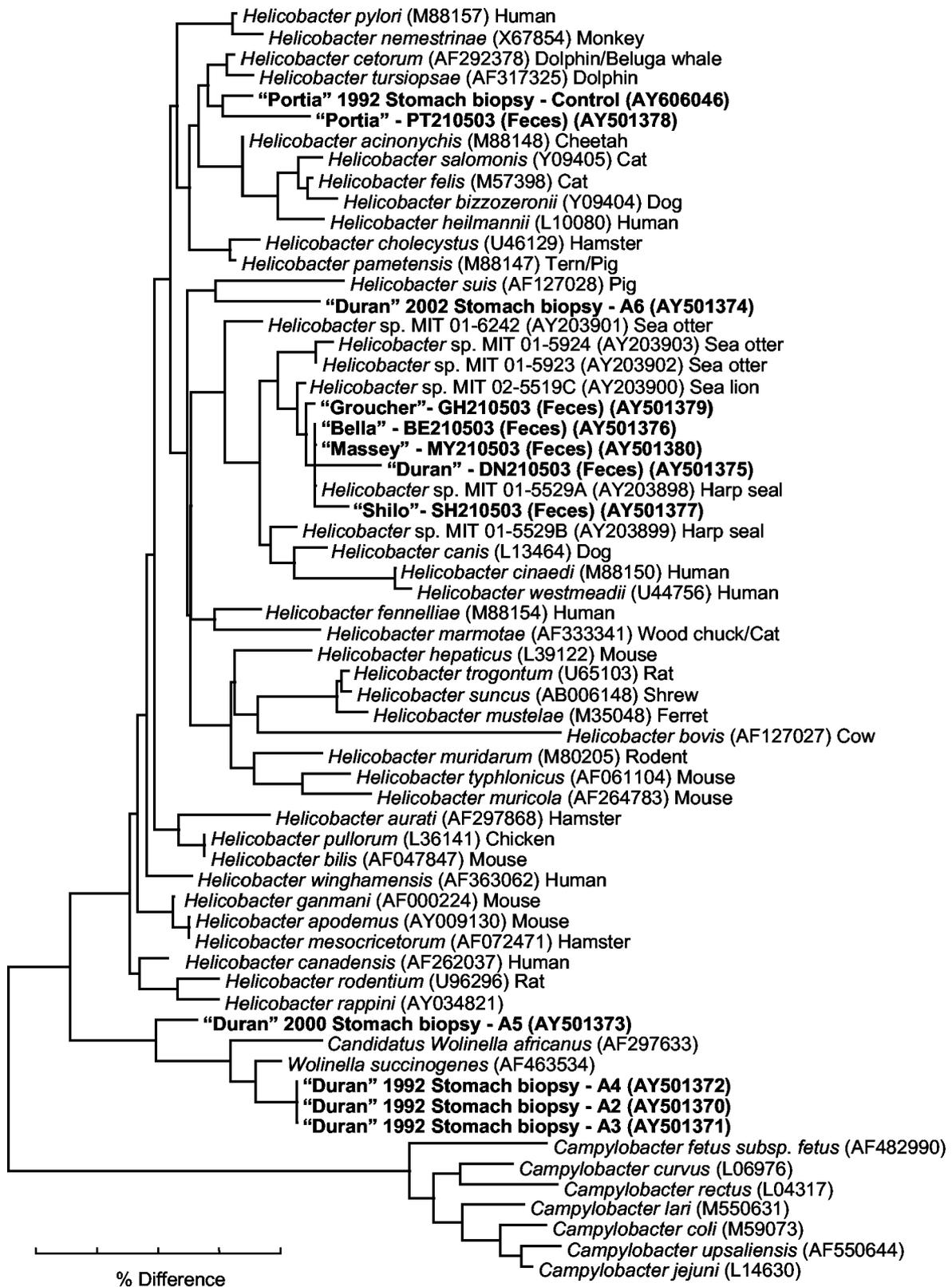


FIG. 6. Neighbor-joining tree demonstrating the 16S rRNA sequence identity among organisms contained within the GenBank database and sequences of the family *Helicobacteraceae* in gut biopsy specimens collected from a male Australian sea lion (Duran) over several years, a biopsy specimen collected from a clinically healthy sea lion (Portia), and fecal material collected from Duran, Portia, and several other cohoused seals in 2003. Code numbers for biopsy samples are included and correspond to those used in the histological images. The scale bar represents a 4% difference in nucleotide sequences. GenBank accession numbers are given in parentheses. Specimens with sequences of interest are shown in boldface characters.

Of particular interest in this study was the detection of a putative novel *Wolinella* species in one of Duran's gastric biopsy specimens (A5), in which gastritis and immense numbers of spiral-shaped organisms were observed. While such organisms (as previously stated) are morphologically consistent with the genus *Helicobacter* (26), it is surprising that given the large numbers of these organisms, a sequence similar to that of *Wolinella* was recovered. Despite the fact that only one species (*W. succinogenes*) has been described to date, another putative novel species (namely, "*Candidatus W. africanus*") was recently detected by PCR in patients with esophageal carcinoma (3). Given the substantial difference in nucleotide sequence (approximately 7 to 12%) from more than 40 *Helicobacter* and *Campylobacter* sequences, it is likely that this sequence represents a novel species. However, as the 16S rRNA sequence is not sufficient to describe a new species (7), other studies clearly are required to isolate and characterize this organism. Nevertheless, given the morphological variation between *Helicobacter* species (26), this putative novel *Wolinella* species may be representative of the spiral-shaped organisms observed in this biopsy specimen and may reflect the potential for more than one *Wolinella* species to infect this host.

A *Helicobacter* sequence type different from those previously detected in the gastric mucosa of wild harp seals exhibiting similar histopathological features of gastritis (17) was also recovered from a gastric biopsy specimen (A6) from Duran. While no spiral-shaped organisms were observed in this (or the control) specimen, such *Helicobacter*-like organisms may not always be detected by standard staining procedures such as HE staining (4). Despite this limitation, its detection in the gut of this host, given the ability of other species to cause similar conditions of disease in other marine mammals (16, 17), is novel and reflects the potential for multiple genera of the family *Helicobacteraceae* to infect this host. Coinfection of a host by closely related taxa has some precedence in the literature and has been reported for *Campylobacter*, *Helicobacter*, and *Wolinella* species (3, 24). While any of these species may have been responsible for the development of gastritis in this animal, they also may have been mere bystanders (3), as species of *Helicobacter*, like those of *Wolinella*, may be nonpathogenic in their natural host (25, 26). Indeed, with the detection of *Helicobacter* species in the gut of a clinically healthy sea lion (Portia) exhibiting no significant pathological features consistent with *Helicobacter* infections in other animals (16, 17), the role of *Helicobacter* in this host requires further examination.

Interestingly, the *Helicobacter* sequence obtained from one of Duran's gastric biopsy specimens was different from that recovered from his feces or feces from the other seals. Moreover, while this sequence was different from that obtained from gastric tissue and feces from the clinically healthy sea lion (Portia), the sequence obtained from feces from Duran and the other seals clustered together with sequences from *Helicobacter* species from other marine mammals. Since gastric *Helicobacter* species are not often detected in the feces of the host (14, 30), such sequences (despite recovery from Duran's ileal biopsy specimen) may have been representative of an enteric species common to both Duran and the other seals. However, since multiple gastric and enteric species may commonly colonize the digestive systems of a wide variety of hosts (11, 26), the recovery of different *Helicobacter* sequence types from gut

biopsy specimens from Portia and Duran may represent the potential for more than one species to colonize the gut of these animals.

Although it is unclear (without further endoscopic examination) whether other seals in this population shared similar gastric *Helicobacter* (or even *Wolinella*) species, it is interesting that only Duran exhibited clinical signs of disease. However, as the relationship between disease and infection is often host specific (6, 13, 21), it is not surprising that animals like Duran may suffer from such infections while other animals like Portia remain asymptomatic. Nevertheless, since clinical disease may be absent in the presence of profound pathological changes (8, 22, 26) or, more importantly, that organisms like *Helicobacter* may establish persistent infections despite activating host defense responses (23), further studies should be directed to the routine endoscopic examination of asymptomatic animals. The extent of these infections may be better elucidated by such studies.

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