Helicobacter acinonyx sp. nov., Isolated from Cheetahs with Gastritis

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Four strains of a novel *Helicobacter* species were isolated from the stomachs of cheetahs (*Acinonyx jubilatus*) with gastritis. These isolates were phenotypically similar to *Helicobacter pylori*. The isolates were gram-negative, spiral bacteria which grew under microaerophilic conditions at 37°C, but not at 25 or 42°C, and produced urease, catalase, oxidase, alkaline phosphatase, and gamma-glutamyl transpeptidase. The isolates did not ferment glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, or arabinose; hydrolyze hippurate or indoxyl acetate; or reduce nitrate. They did not produce H₂S from triple sugar iron agar, and they did not grow in the presence of 1.0% glycine or 1.5% NaCl. They were resistant to nalidixic acid and sensitive to cephalothin and metronidazole. Cells were typically 0.3 by 2.0 µm and possessed tufts of two to five sheathed, monopolar flagella. The G+C content of strain 90-119 was 30 mol%. Cluster analysis of densitometry scans of polyacrylamide protein gels revealed more than 70% similarity of the cheetah isolates to *H. pylori*, less than 60% similarity to *Helicobacter felis*, and less than 50% similarity to *Helicobacter mustelae*. Complete 16S rRNA sequences were determined for two of the cheetah isolates. Phylogenetic analysis was performed by comparing the cheetah sequences to those of 19 reference strains, including *H. pylori*, *H. felis* (two strains), *H. mustelae*, *Helicobacter muridarum*, “*Flexispira rappini*,” *Wolinella succinogenes*, *Campylobacter coli*, *Campylobacter concisus*, *Campylobacter curvus*, *Campylobacter fetus*, *Campylobacter hyointestinalis*, *Campylobacter jejuni*, *Campylobacter lari*, *Campylobacter rectus*, *Campylobacter sputorum* subsp. *bubulus*, a *Campylobacter* sp. (pig isolate), [*Bacteroides*] *gracilis*, and [*Bacteroides*] *ureolyticus*. The 16S rRNA sequences for 13 of the 19 reference species have not previously been reported. Phylogenetic analysis demonstrated that the cheetah isolates were most closely related to *H. pylori* (97.4% similarity), *H. felis* (96.1% similarity), and *H. mustelae* (93.4% similarity). On the basis of these findings, we propose that these isolates represents a novel species of *Helicobacter*, which we designate *Helicobacter acinonyx*. The type strain is 90-119 (CCUG 29263, ATCC 51101).

MATERIALS AND METHODS

The genus *Helicobacter* currently comprises seven species: *Helicobacter pylori* (25), *H. mustelae* (9–11, 14, 30), *H. felis* (21–23, 30), *H. muridarum* (24), *H. cinaedi* (8, 35, 36), *H. fennelliae* (8, 35, 36), and *H. nemestrinae* (2). Strains representing six additional *Helicobacter* species have been identified by rRNA-DNA hybridization (34) and 16S rRNA sequencing (5). These include three species isolated from bird feces (29); “*Flexispira rappini*,” isolated from human and ovine sources (1, 4); CLO-3, isolated from humans (8, 35); 90-266-11, a strain isolated from ferret feces that differs from *H. mustelae* (13); and CCUG 29176, a strain isolated from human blood. Many of the *Helicobacter* species cause chronic gastritis in their natural hosts: *H. pylori* in humans (25), *H. mustelae* in ferrets (11), and *H. felis* in dogs (23). *H. felis* also induces gastritis when inoculated into germfree mice and rats (12, 21), as does *H. pylori* when given per os to germfree piglets and dogs (19, 31). In this paper we describe a novel *Helicobacter* sp. based on four isolates from captive cheetahs with chronic gastritis. These isolates are biochemically and culturally similar to previously described *Helicobacter* species but are clearly differentiated from known *Helicobacter* species by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis protein profile, G+C content, and 16S rRNA sequence. We propose that the organism be named *Helicobacter acinonyx*.

Bacterial strains and culture conditions. The strain names, sources, and accession numbers for the organisms examined in this study are listed in Table 1. The isolation of *Helicobacter* species strains 90-119, 90-548, 90-736, and 90-1908 from gastric biopsies of four captive cheetahs (*Acinonyx jubilatus*) was previously described (6). Unless otherwise stated, all bacteria were grown on tryptic soy agar (BBL, Cockeysville, Md.) with 5% sheep blood (blood agar plates) and incubated for 3 days at 37°C in an atmosphere of 5% O₂, 10% CO₂, and 85% N₂. Growth at 25 and 42°C was determined on blood agar plates under microaerophilic conditions. Aerobic and anaerobic growth was determined at 37°C. Anaerobiosis was achieved with a GasPak jar and anaerobic generator envelope (BBL). Growth on 1.0% glycine and 1.5% NaCl was determined with brucella agar (Difco Laboratories, Detroit, Mich.) containing 10% fetal calf serum (NaCl) or 10% sheep blood (glycine) and the appropriate additive. Antibiotic sensitivities were determined by using blood agar plates. The surfaces of the plates were inoculated with a swab which had been dipped in a heavy suspension of bacteria. Antibiotic discs were placed on the plates. Strains were considered sensitive if there was a zone of inhibition of 2 cm or more after 3 days of incubation. Antibiotics used were purchased from BBL except for metronidazole (Unipath, Columbia, Md.). The antibiotics used were cephalothin (30 µg), nalidixic acid (30

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TABLE 1. Sources and accession numbers of strains studied

Organism ^a	Strain ^b	Source ^c	Other source(s) and strain no. ^d	GenBank no. ^e
<i>Helicobacter acinonyx</i>	90-119	Eaton	CCUG 29263 ^T , ATCC 51101	M88148 ^f
<i>Helicobacter acinonyx</i>	90-548	Eaton	ATCC 51102	M37642
<i>Helicobacter acinonyx</i>	90-736	Eaton	ATCC 51103	
<i>Helicobacter acinonyx</i>	90-1908	Eaton	ATCC 51104	M88149 ^f
<i>Helicobacter felis</i>	CS1	Lee	ATCC 49179 ^T	M37643
<i>Helicobacter felis</i>	DS3	Lee		M35048
<i>Helicobacter mustelae</i>	R85-13-6P	Fox	ATCC 43772 ^T	M80205
<i>Helicobacter muridarum</i>	ST1	Lee	ATCC 49282 ^T , CCUG 29262 ^T	M88157 ^f
<i>Helicobacter pylori</i>	ATCC 43504 ^T	ATCC		M88137 ^f
" <i>Flexispira rappini</i> "	NADC 1893	Bryner	ATCC 43966	M88138 ^f
" <i>Flexispira rappini</i> "	NADC 1937	Bryner	ATCC 43879, CCUG 23435	M88159 ^f
<i>Wolinella succinogenes</i>	ATCC 29543 ^T	ATCC		L04312 ^f
<i>Campylobacter coli</i>	CCUG 11283 ^T	CCUG		L04322 ^f
<i>Campylobacter concisus</i>	FDC 484 ^T	Tanner	ATCC 33237 ^T	L04313 ^f
<i>Campylobacter curvus</i>	ATCC 35224 ^T	ATCC		M65012
<i>Campylobacter fetus</i> subsp. <i>fetus</i>	ATCC 27374 ^T	ATCC		M65010
<i>Campylobacter hyointestinalis</i>	ATCC 35217 ^T	ATCC		L04315 ^f
<i>Campylobacter jejuni</i>	CCUG 112844	CCUG		L04316 ^f
<i>Campylobacter lari</i>	CCUG 23947 ^T	CCUG		L04317 ^f
<i>Campylobacter rectus</i>	ATCC 33238 ^T	ATCC		L04318 ^f
<i>Campylobacter species</i>	40-6AT	Morgan		L04319 ^f
<i>Campylobacter sputorum</i> subsp. <i>bubulus</i>	ATCC 33491	ATCC		L04320 ^f
[<i>Bacteroides</i>] <i>gracilis</i>	ATCC 33236 ^T	ATCC		L04321 ^f
[<i>Bacteroides</i>] <i>ureolyticus</i>	ATCC 33387 ^T	ATCC		

^a Organisms are listed according to current nomenclature. Square brackets indicate that an organism does not belong in the genus indicated.

^b Abbreviations for culture collections: ATCC, American Type Culture Collection; CCUG, Culture Collection, University of Göteborg; FDC, Forsyth Dental Center, Boston, Mass.; NADC, National Animal Disease Center, Ames, Iowa.

^c Strains were obtained directly from the indicated culture collection (abbreviated as shown above) or from individuals, as follows: J. H. Bryner, National Animal Disease Center; K. Eaton, Department of Veterinary Pathobiology, Ohio State University, Columbus; J. G. Fox, Massachusetts Institute of Technology, Cambridge, Mass.; A. Lee, School of Microbiology and Immunology, University of New South Wales, Sydney, New South Wales, Australia; D. R. Morgan, The Procter and Gamble Co., Cincinnati, Ohio; and A. R. C. Tanner, Forsyth Dental Center.

^d Culture collection sources for strains obtained from individuals. Culture collection abbreviations are as shown above.

^e 16S rRNA sequences for these strains are available for electronic retrieval from GenBank under the indicated accession numbers. Through cross distribution of data bases, these sequences should also be available from European and Japanese data bases.

^f Sequence deposited with GenBank for this publication.

µg), metronidazole (5 µg), penicillin (2 U), ampicillin (10 µg), nitrofurazone (100 µg), sulfamethoxazole (33.75 µg) with trimethoprim (1.25 µg), erythromycin (15 µg), vancomycin (30 µg), gentamicin (10 µg), chlortetracycline (30 µg), and chloramphenicol (30 µg).

Electron microscopy. Cells were negatively stained with 1% phosphotungstic acid (pH 6.5) for 15 to 20 s. Specimens were examined with a Jeol JEM-1200EX transmission electron microscope operating at 100 kV.

Biochemical characterization. Bacteria were grown in brucella broth (Difco) with 10% fetal bovine serum. Bacteria were harvested by centrifugation, washed in phosphate-buffered saline, counted with a hemacytometer, and adjusted to 1.0×10^9 bacteria per ml. Production of urease was determined by observing a color change upon inoculation of urea broth (BBL), production of catalase was determined by inoculation of 3% H₂O₂ and looking for bubbles, and production of oxidase was determined by assessing the ability to oxidize *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride (Spotstest; Difco). Monosaccharide fermentation, H₂S production, and nitrate reduction were tested with API 20E kits (Analytab, Plainview, N.Y.) according to the manufacturer's instructions (7). In addition, cultures were inoculated into triple sugar iron slants (BBL) and incubated overnight in 10% CO₂ at 37°C. Aminopeptidases, alkaline phosphatase, acid phosphatase, and phosphohydrolase tests were performed with API ZYM (16) and An-IDENT (16, 37) kits (Analytab). Gamma-glutamyl transpeptidase was measured by adding 100 µl of suspended bacteria (1×10^9

bacteria per ml) to 100 µl of 0.2% gamma-glutamyl-beta-naphthylamide in 50 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) (pH 7.5) buffer in a 96-well microtiter plate. Following 4 h of incubation at 37°C, the reaction was developed with 1 drop of Cinnamaldehyde Reagent (An-IDENT; Analytab Products). The indoxyl acetate hydrolysis test was performed by the disc method of Hodge et al. (15). Positive reactions were indicated by the appearance of a green or blue color within 20 min. The hippurate hydrolysis test was performed by the method of Hwang and Ederer (17).

TABLE 2. Sequencing primers

Primer	Positions	Sequence ^a
1	159-175	5'-GTATTAATCACCGTTTC-3'
2	344-358	5'-ACTGCTGCCTCCCGT-3'
3	519-536	5'-GWATTACCGCGGCKGCTG-3'
4	786-803	5'-CTACCAGGGTATCTAATC-3'
5	907-926	5'-CCGTCAATTCTTTT-3'
6	951-964	5'-TCGAATTAAACCAC-3'
7	962-985	5'-GGTAAGGTTCTTCGYGTATCTTCG-3'
8	1096-1113	5'-GGTTGCGCTCGTTGCGGG-3'
9	1392-1406	5'-ACGGGCGGTGTGTRC-3'
10	1493-1513	5'-TACGGYTACCTGTTACGACT-3'

^a The sequences are complementary to 16S rRNA at the positions listed (*E. coli* numbering system). Base codes are standard International Union of Biochemistry codes for bases and ambiguity.

FIG. 1. (A) Electron micrograph of *H. acinonyx* showing short, spiral morphology and polar tuft of flagella. Bar = 0.5 μ m. (B) Electron micrograph of flagellum of *H. acinonyx* showing flagellar sheath (arrow). Bar = 0.1 μ m.

Protein profiles. Protein profiles for the cheetah isolates and reference strains were determined by Coomassie blue staining of proteins separated on SDS-12% polyacrylamide gels (6). Densitometry scans of SDS gels were performed with an Ultrosan XL laser densitometer and analyzed with custom-designed software (26).

G+C content. The G+C content of DNA was determined

by thermal denaturation analysis as previously described (24). DNA isolated from *Escherichia coli* K-12 was used for control determinations.

16S rRNA sequencing. rRNAs were isolated and partially purified by a modification of the procedure of Pace et al. (27) as previously described (28). rRNA sequences were determined by using a modification of the standard Sanger

FIG. 2. (A) Polyacrylamide gel protein profile of *H. acinonyx* and related species. Lanes 1 and 20, protein size markers; lanes 2 through 6 and 15 through 19, *H. pylori*; lane 7, *H. felis*; lanes 8 through 11, *H. acinonyx*; lanes 12 through 14, porcine *Campylobacter* species strains. (B) Phylogenetic tree derived from cluster analysis of densitometry scans of the gel shown in panel A. Horizontal scale indicates percent similarity.

dideoxy chain termination technique (20). Primers complementary to conserved regions of the 16S rRNA sequence were elongated by using avian myeloblastosis virus reverse transcriptase (28). The eight primers used to obtain nearly complete sequences for *H. acinonyx* and reference species are given in Table 2. For most of the species examined in this study, primers 2 through 6 and 8 through 10 were used. Primers 6 and 7 were used to obtain sequence information 5' to a hard stop at *E. coli* position 970. Primer 6 was used in early sequencing studies and was subsequently replaced by primer 7. Primer 1 was used to obtain sequence information 5' to a hard stop at *E. coli* position 210 in some *Helicobacter* species.

16S rRNA data analysis. A program set for data entry,

editing, sequence alignment, secondary-structure comparison, similarity matrix generation, and dendrogram construction for 16S rRNA data was written in Microsoft QuickBAS for use on IBM PC-AT and compatible computers. RNA sequences were entered and aligned as previously described (28). Similarity matrices were constructed from the aligned sequences by using only those sequence positions for which 90% of the strains had data. The similarity matrices were corrected for multiple base changes by the method of Jukes and Cantor (18). Phylogenetic trees were constructed by the neighbor-joining method (32).

GenBank accession numbers. GenBank accession numbers for organisms examined in this report are given in Table 1. Strains not previously recorded are *H. acinonyx*, *H. pylori* (ATCC 43504^T), "*F. rappini*," *Wolinella succinogenes* (ATCC 29543^T), *Campylobacter coli* (CCUG 11283^T), *Campylobacter concisus* (ATCC 33237^T), *Campylobacter curvus* (ATCC 35224^T), *Campylobacter jejuni* (CCUG 112844), *Campylobacter lari* (CCUG 23947^T), *Campylobacter rectus* (ATCC 33238^T), *Campylobacter sputorum* subsp. *bubulus* (ATCC 33491), a *Campylobacter* sp. (pig isolate, 40-6AT), [*Bacteroides*] *gracilis* (ATCC 33236^T), and [*Bacteroides*] *ureolyticus* (ATCC 33387^T).

RESULTS AND DISCUSSION

Morphologic, ultrastructural, and growth characteristics. *H. acinonyx* cells were short (0.3 by 1.5 to 2 μ m), spiral, gram-negative rods. They possessed tufts of two to five monopolar sheathed flagella (Fig. 1). Cells were motile. No periplasmic fibers, as are seen in *H. felis*, *H. muridarum*, and "*F. rappini*," were observed. Spherical forms 2 to 4 μ m in diameter were observed in older cultures. *H. acinonyx* strains grew under microaerophilic conditions at 37°C but not at 25 or 42°C. No growth was seen on blood agar plates under aerobic or anaerobic conditions. These strains did not grow on brucella agar plates containing 1.0% glycine or 1.5% NaCl.

Biochemical characteristics. *H. acinonyx* strains produced oxidase, catalase, alkaline phosphatase, acid phosphatase, phosphohydrolase, urease, gamma-glutamyl transpeptidase, and arginine beta-naphthylamide aminopeptidase. Leucine and histidine beta-naphthylamides were weakly hydrolyzed. *H. acinonyx* did not hydrolyze hippurate or produce acid from glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, or arabinose. H_2S was not produced. Proline, pyroglutamic acid, tyrosine, alanine, phenylalanine, and glycine beta-naphthylamides were not hydrolyzed. Strains of *H. acinonyx* were sensitive to metronidazole and cephalothin and resistant to nalidixic acid (no zone of inhibition). In addition, they were sensitive to penicillin, ampicillin, nitrofurazone, erythromycin, gentamicin, chlortetracycline, and chloramphenicol. They were resistant to sulfamethoxazole with trimethoprim and to vancomycin. Comparison of the biochemical characteristics of *H. acinonyx* with those of related organisms is shown in Table 3. The essential phenotypic distinctions were in size, morphology, and G+C content as well as 16S rRNA sequence (see below).

Total bacterial protein profile. Laser densitometry scanning of SDS-polyacrylamide gels indicated more than 70% similarity between *H. acinonyx* and *H. pylori* (Fig. 2). In contrast, *H. acinonyx* is less than 60% similar to *H. mustelae* and less than 50% similar to *H. felis*. Strains of a porcine gastric campylobacter are even less similar. The porcine campylobacter will be described in a subsequent report. Pre-

TABLE 3. Phenotypic characteristics of selected *Helicobacter* and *Campylobacter* species^a

Characteristic	<i>H. acinonyx</i>	<i>H. pylori</i>	<i>H. felis</i>	<i>H. mustelae</i>	<i>H. muridarum</i>	<i>H. nemes-</i> <i>trinae</i>	<i>H. cinaedi</i>	<i>H. fennelliae</i>	" <i>F. rappini</i> "	<i>W. succino-</i> <i>genes</i>	<i>C. fetus</i>	<i>C. jejuni</i>
Oxidase production	4/4 ^b	+	+	+	+	+	+	+	+	+	+	+
Catalase production	4/4	+	+	+	+	+	+	+	+	+	+	+
Alkaline phosphatase production	4/4	+	+	+	+	ND	+	+	+	+	+	+
Urease production	4/4	+	+	+	+	+	+	+	+	+	+	+
Gamma-glutamyl transpeptidase production	4/4	+	+	+	+	ND	+	+	+	+	+	+
Hippurate hydrolysis	0/4	-	-	-	-	ND	-	-	ND	-	-	+
Indoxyl acetate hydrolysis	0/4	-	-	-	-	ND	-	-	ND	-	-	+
lys												
Nitrate reduction	0/4	-	+	+	-	-	+	-	-	+	-	-
Growth on:												
1.0% glycine	0/4	-	-	-	-	-	+	+	ND	-	+	+
1.5% NaCl	0/4	-	-	-	-	-	-	-	ND	-	-	Variable
Growth at:												
25°C	0/4	-	-	-	-	-	-	-	-	-	-	-
42°C	0/4	-	+	+	+	+	-	-	+	+	+	+
Sensitivity to:												
Cephalothin	S (4/4)	S	S	R	R	S	I	S	R	R	S	R
Nalidixic acid ^c	R (4/4)	R	R	S	R	R	ND	ND	R	R	R	S
Metronidazole	S (4/4)	S	S	S	ND	S	ND	ND	R	ND	ND	R
Flagella												
Sheath	+	+	+	+	+	+	+	+	+	+	+	-
No.	2-5	4-8	14-20	4-8	10-14	4-8	2	2	10-20	1	1	1
Distribution	Polar	Polar	Bipolar	Peritrichous	Bipolar	Polar	Bipolar	Bipolar	Bipolar	Polar	Polar	Polar
Periplasmic fibers	-	-	+	+	+	+	-	-	+	-	-	-
Size (µm)	0.3 × 1.5-2.0	0.5 × 3.0-5.0	0.4 × 5.0-7.5	0.5 × 2.5-5.0	0.5 × 3.5-5.0	0.2 × 2.0-5.0	0.3-0.5 × 1.5-5.0	0.3-0.5 × 1.5-5.0	0.5 × 6.5	0.5 × 2.0	0.5 × 5.0	0.5 × 5.0
G+C content (mol%)	30	35-37	42	36	34	24	37-38	35	34	47	34	30-33
Source of data (reference)	This study	This study	This study	This study	This study	2	8, 35	8, 35	1, 4	This study, 34	This study, 33	This study, 33

^a Abbreviations: +, positive; -, negative; S, sensitive; R, resistant; I, intermediate; ND, not done.
^b Number of positive strains/number tested.
^c Strains which were resistant to nalidixic acid showed no zone of inhibition.

FIG. 3. Aligned 16S rRNA sequences for *H. acinonyx* 90-119 (Hac), *H. pylori* ATCC 43504^T (Hpy), and *E. coli* (Ec). The numbering system is that of *E. coli* (3). The sequences are reported by using the International Union of Biochemistry single-letter code for nucleotide bases and ambiguities. Lowercase letters indicate some uncertainty in the base identity. Dashes indicate gaps inserted for alignment of sequences, and dots indicate regions that were not sequenced.

vious studies indicate that strains with 90% similarity are identical (26).

16S rRNA sequence. We determined approximately 95% of the total 16S rRNA sequence for strains 90-119 and 90-1908

and for the reference strains listed in Table 1. The sequences for the two cheetah isolates were identical. In Fig. 3, the 16S rRNA sequence of strain 90-119 is aligned with sequences for *H. pylori* and *E. coli*. Sequences for the remaining reference strains are available for electronic retrieval from GenBank under the accession numbers given in Table 1. A detailed discussion of these reference sequences is the subject of a manuscript in preparation on the phylogeny of the genera *Campylobacter* and *Helicobacter*. A similarity matrix comparing *H. acinonyx* with 19 related species is given in Table 4. This matrix is based on sequence comparisons at 1,392 base positions. *H. acinonyx* is most closely related to *H. pylori* (97.4% similarity) and the two *H. felis* strains (96.1 and 96.4% similarities). There are approximately 40 base differences between *H. acinonyx* and *H. pylori* 16S rRNA sequences which allow the design of DNA probes to easily differentiate these organisms. The probe 5'-CGTAGGAATATCTGGTATTAATCACCG-3' (*E. coli* positions 163 to 190) to *H. acinonyx* should not recognize *H. pylori*. *H. acinonyx* has four single-base signatures which differentiate it from other helicobacters: C instead of U at positions 239, 1297, 1336, and 1453. A phylogenetic tree determined from the similarity data is shown in Fig. 4. *H. acinonyx*, *H. pylori*, and *H. felis* form a cluster that is separated from the other *Helicobacter* species (less than 94% similarity). The two "*F. rappini*" strains examined are identical to one another and branch within the helicobacter

TABLE 4. Similarity matrix based on 16S rRNA sequences^a

Organism	% Similarity and % difference compared with ^b :																			
	Ha	Hp	HfD	HfC	Hms	Hmr	Fr	Ws	Bu	Cr	Bg	Ccr	Ccn	Cs	Cf	Ch	Csp	Cco	Cj	Cl
Ha	--	97.4	96.1	96.4	93.4	92.4	92.4	90.4	83.9	83.6	83.0	84.8	84.7	85.2	84.6	83.8	83.4	84.5	84.8	84.6
Hp	2.7	--	95.3	95.4	93.8	93.0	93.1	90.7	84.1	84.1	83.9	85.2	85.1	85.7	85.2	84.6	84.1	84.9	85.2	85.1
HfD	4.0	4.9	--	99.4	93.0	92.5	92.4	90.2	83.8	83.1	83.0	84.2	84.7	85.0	84.9	84.0	83.9	84.1	84.7	84.8
HfC	3.6	4.7	0.6	--	93.4	92.6	92.6	90.1	83.6	83.1	82.9	84.2	84.6	85.1	84.8	83.8	83.7	84.1	84.6	84.8
Hms	6.9	6.5	7.3	6.9	--	95.7	95.8	93.7	85.7	84.9	84.4	85.8	86.0	87.4	86.0	85.6	85.3	86.0	86.5	86.7
Hmr	8.0	7.3	7.9	7.8	4.4	--	96.2	93.3	84.8	84.9	84.1	85.3	86.3	86.7	85.7	85.3	85.0	85.5	85.6	85.8
Fr	8.0	7.3	8.0	7.8	4.3	3.9	--	93.1	86.3	86.1	85.5	86.3	87.4	88.4	86.9	86.3	85.9	86.9	86.7	86.9
Ws	10.3	9.9	10.5	10.6	6.6	7.0	7.2	--	85.8	86.2	85.3	86.9	86.4	86.3	86.0	85.4	85.3	85.8	86.1	86.3
Bu	18.2	17.8	18.3	18.5	15.8	16.9	15.1	15.7	--	92.0	91.5	90.8	91.8	92.1	92.8	92.9	92.1	92.9	93.1	92.5
Cr	18.5	17.9	19.1	19.1	16.9	16.8	15.3	15.2	8.5	--	95.4	96.4	95.8	95.1	93.8	92.9	92.7	92.7	92.3	92.1
Bg	19.3	18.1	19.2	19.4	17.5	17.9	16.1	16.4	9.1	4.8	--	94.4	94.7	93.9	93.9	93.0	91.6	91.3	91.1	90.9
Ccr	16.9	16.5	17.8	17.8	15.7	16.4	15.1	14.4	9.8	3.6	5.9	--	96.9	95.2	94.4	93.5	93.9	92.8	92.4	91.9
Ccn	17.2	16.7	17.1	17.2	15.5	15.1	13.8	15.0	8.6	4.3	5.5	3.2	--	95.1	95.5	94.5	93.9	93.8	93.4	93.6
Cs	16.5	15.8	16.8	16.6	13.8	14.7	12.6	15.1	8.4	5.1	6.3	5.0	5.1	--	93.3	92.5	92.9	92.5	92.0	91.8
Cf	17.2	16.4	16.8	17.0	15.5	15.8	14.3	15.4	7.6	6.5	6.3	5.8	4.6	7.0	--	98.1	95.5	95.0	94.3	94.1
Ch	18.2	17.2	18.0	18.2	15.9	16.4	15.1	16.3	7.5	7.5	7.3	6.8	5.7	7.9	1.9	--	95.8	95.2	94.6	94.4
Csp	18.8	17.8	18.1	18.4	16.3	16.7	15.6	16.4	8.4	7.6	8.9	6.4	6.4	7.5	4.6	4.3	--	95.7	95.0	94.6
Cco	17.4	16.9	17.8	17.9	15.5	16.1	14.4	15.7	7.4	7.7	9.3	7.5	6.4	7.9	5.2	4.9	4.4	--	98.1	97.2
Cj	17.0	16.5	17.2	17.2	14.8	16.0	14.6	15.4	7.2	8.1	9.4	8.0	6.9	8.4	5.9	5.6	5.1	1.9	--	98.3
Cl	17.2	16.7	16.9	17.0	14.6	15.8	14.4	15.2	7.9	8.4	9.7	8.6	6.7	8.7	6.2	5.9	5.6	2.8	1.7	--

^a Abbreviations: Ha, *H. acinonyx*; Hp, *H. pylori*; HfD, *H. felis* DS3; HfC, *H. felis* CS1; Hms, *H. mustelae*; Hmr, *H. muridarum*; Fr, "*F. rappini*"; Ws, *W. succinogenes*; Bu, [*B.*] *ureolyticus*; Cr, *C. rectus*; Bg, [*B.*] *gracilis*; Ccr, *C. curvus*; Ccn, *C. concisus*; Cs, *C. sputorum*; Cf, *C. fetus*; Ch, *C. hyointestinalis*; Csp, *Campylobacter* sp.; Cco, *C. coli*; Cj, *C. jejuni*; Cl, *C. lari*.

^b Numbers above the diagonal are uncorrected percentages of similarity. Numbers below the diagonal are percentages of difference corrected for multiple base changes by the method of Jukes and Cantor (18).

cluster. By 16S rRNA analysis, they appear to belong in the genus *Helicobacter*. Studies are in progress to determine whether strains described as "*F. rappini*" are members of a single, genetically homogeneous species. The spiral organism isolated by Philips and Lee from rodent intestinal mucosa was once considered an "*F. rappini*" strain (4) but is now recognized as *H. muridarum* (24). Previous arguments for excluding "*F. rappini*" from the genus *Helicobacter* (14) have been diluted by the inclusion of *H. muridarum*, which is morphologically identical to "*F. rappini*," and *H. cinaedi*, which, like "*F. rappini*," fails to produce alkaline phosphatase.

G+C content. The G+C content of *H. acinonyx* was 29.9 ± 0.4 mol%. This value differentiates *H. acinonyx* from the other seven described *Helicobacter* species.

Description of *H. acinonyx* sp. nov. *Helicobacter acinonyx* (acinonyx, L. gen. n. of the species *Acinonyx jubiliatus*, from which it was isolated) is a short, spiral, gram-negative rod, 0.3 by 1.5 to 2.0 µm. Cells are motile and possess tufts of two to five sheathed monopolar flagella. No endospores are produced. Microaerophilic growth occurs at 37°C but not at 25 or 42°C. Growth does not occur aerobically or anaerobically. Acid is not produced from glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, or arabinose. Oxidase, catalase, alkaline phosphatase, acid phosphatase, phosphohydrolase, urease, gamma-glutamyl transpeptidase, and arginine beta-naphthylamide aminopepti-

dase are produced. Proline, pyroglutamic acid, tyrosine, alanine, phenylalanine, and glycine beta-naphthylamide aminopeptidase are not detected. Hippurate and indoxyl acetate are not hydrolyzed. H₂S is not produced. Nitrate is not reduced. Growth does not occur in the presence of 1.0% glycine or 1.5% NaCl. *H. acinonyx* is resistant to nalidixic acid and sensitive to cephalothin and metronidazole. In addition, it is sensitive to penicillin, ampicillin, nitrofurazone, erythromycin, gentamicin, chlortetracycline, and chloramphenicol and resistant to sulfamethoxazole with trimethoprim and to vancomycin. *H. acinonyx* was isolated from the gastric mucosa of cheetahs. Four strains (90-119, 90-548, 90-736, and 90-1908) have been isolated and are available from The American Type Culture Collection (ATCC 51101, 51102, 51103, and 51104, respectively). The type strain, 90-119, has a G+C content of 30 mol%. It is deposited in the Culture Collection of the University of Göteborg, Göteborg, Sweden (CCUG 29263).

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