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Misidentifying Helicobacters: the *Helicobacter cinaedi* Example

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Whole-cell protein electrophoresis and biochemical examination by means of a panel of 64 tests were used to identify 14 putative helicobacters to the species level. The results were confirmed by means of DNA-DNA hybridization experiments and were used to discuss misidentification of helicobacters based on 16S rRNA gene sequence data. The data indicated that comparison of near-complete 16S ribosomal DNA sequences does not always provide conclusive evidence for species level identification and may prove highly misleading. The data also indicated that "*Helicobacter westmeadii*" is a junior synonym of *Helicobacter cinaedi* and that *Helicobacter* sp. strain Mainz belongs to the same species. *H. cinaedi* occurs in various animal reservoirs, including hamsters, dogs, cats, rats, and foxes. Appropriate growth conditions and identification strategies will be required to establish the genuine significance of this widely distributed *Helicobacter* species.

The cultivation of *Helicobacter pylori* from the human gastric mucosa in the early 1980s (33) and the demonstration of its relationship to gastritis, peptic ulcer disease, and gastric neoplasia triggered a series of studies of the ecology and role of *Helicobacter*-like organisms in a variety of hosts. At present, 18 validly named species and two candidate species (3, 4) have been isolated from gastric or enteric samples of a variety of hosts, including humans, hamsters, rats, mice, ferrets, pigs, poultry, wild birds, cats, dogs, monkeys, and cheetahs. Several other *Helicobacter* taxa remain unnamed or have not been properly described in accordance with internationally accepted rules of nomenclature, and therefore their names have not been validated (6, 9, 13, 29, 30). The biochemical inertness of all *Campylobacter*-like organisms, including helicobacters, plays a major role in influencing the identification strategies of clinical laboratories. Classical phenotypic tests routinely used for the identification of clinical bacteria often yield negative or variable results within species. Problems associated with phenotypic identification have led to sequence analysis of rRNA genes (in particular 16S) as an increasingly popular alternative approach for identification of new isolates. Strains have been identified as novel species, primarily because of supposedly sufficient differences in 16S ribosomal DNA (rDNA) sequence similarity to known species (for example, reference 13), or as well-established species, again primarily based on the percentage of similarity of 16S rDNA sequence (for example, references 7 and 28).

In the present study, we describe the identification and characterization of 14 *Helicobacter cinaedi* isolates obtained from various hosts, including humans, dogs, foxes, and a rat, by using different phenotypic and genotypic approaches and comment on the pitfalls of the 16S rDNA sequence approach. These 14 strains included isolates described in this journal as "*Helicobacter westmeadii*" sp. nov. (30) and as *Helicobacter* sp. nov. strain Mainz (13).

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MATERIALS AND METHODS

Strains. Three *H. cinaedi* reference strains (LMG 7543^T, LMG 8770, and LMG 9071) from the original study by Totten et al. (29) and five additional strains (LMG 8558, LMG 8559, LMG 9072, LMG 9153, and LMG 9357), characterized in a previous polyphasic taxonomic study (31), were used as references (Table 1). Fourteen recent isolates obtained from the feces of dogs, foxes, and a rat and from human blood or feces were studied; the isolates were from Finland ($n = 6$), Belgium ($n = 2$), Sweden ($n = 2$), Australia ($n = 2$), Scotland ($n = 1$), and Germany ($n = 1$) (Table 1).

Representative strains of all presently named *Helicobacter* species were obtained from the Culture Collection, University of Göteborg, Department of Clinical Bacteriology, Göteborg, Sweden (CCUG) or the Bacteria Collection, Laboratorium voor Microbiologie Gent, Universiteit Gent, Ghent, Belgium (LMG) and were included as references.

PAGE of whole-cell proteins. All 14 isolates were grown on Mueller-Hinton agar (catalog no. CM 337; Oxoid, Ltd., Basingstoke, United Kingdom) supplemented with 5% (vol/vol) horse blood and were incubated at 36 to 37°C in a microaerobic atmosphere containing approximately 5% O_2, 3.5% CO_2, 7.5% H_2, and 84% N_2. Whole-cell protein extracts were prepared, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (PAGE) was performed as described before (26). Whole-cell protein profiles of *H. cinaedi* reference strains and of type and reference strains of other *Helicobacter* species were available from previous studies. The densitometric analysis, normalization, and interpolation of the protein profiles and numerical analysis were performed with the GelCompar software package version 4.2 (Applied Maths, Kortrijk, Belgium). The profiles were recorded and stored on an IBM PC-compatible computer. The similarities between all pairs of traces were expressed by the Pearson product moment correlation coefficient presented below as percentages of similarity for convenience.

Phenotypic analysis. All 14 isolates and reference strain LMG 9357 (not examined before by this scheme) were grown on 5% (vol/vol) calf blood agar for 3 days under microaerobic conditions, as described previously (19). A total of 64 phenotypic characters were determined using methods described previously (19–22). The results were compared with data for 37 *Campylobacter* taxa in a probability matrix using computer-assisted methods.

Dot blot DNA-DNA hybridizations. DNA was isolated by the method of Pitcher et al. (25), with modifications described before (14). Dot blot DNA-DNA hybridizations were performed as described before (10, 14). The DNA of *H. cinaedi* LMG 7543^T was used as a probe. DNAs of the following strains were hybridized with the LMG 7543^T probe: LMG 7543^T, LMG 8559, and LMG 8558 (the three reference strains) and R-927, R-2971, R-2977, R-2981, R-2983, R-2991, and R-5759. "*Flexispira rappini*" CCUG 23435 and *Helicobacter canis* CCUG 19561 served as negative controls. Aliquots of 0.5, 5, and 50 ng of chromosomal DNA were spotted onto the membranes for each hybridization experiment.

16S rDNA sequencing. The primers and methods used for DNA extraction, PCR amplification, and direct, automated sequencing of 16S rRNA genes were as described previously (18), except that primer 1492r (5′-TACGGYTACCTT GTTACGACTT) was used in place of 1392r for the initial PCR amplification and subsequent sequencing of the PCR product. This allowed sequences to be obtained (both strands) over ~95% (compared to ~90%) of the 16S rRNA gene. In addition, the *Taq* polymerase and PCR buffer (1.5 mM final $MgCl_2$ concentration) were from Boehringer (Mannheim, Germany). The consensus sequence

TABLE 1. *H. cinaedi* strains examined

Our no.	Other strain no.	Depositor[a]	Source
R-2971	KJ457		Blue fox, feces (Finland)
R-2977	KJ459		Blue fox, feces (Finland)
R-2981	KJ465A		Blue fox, feces (Finland)
R-2982	KJ465B		Blue fox, feces (Finland)
R-2983	KJ412		Dog, feces (Finland)
R-2991	KJ425		Dog, feces (Finland)
R-3026	CCUG 36876	CCUG	Rat, feces (Sweden)
R-4792	ADN 0413	M. MacLennan	Dog, feces (Scotland)
R-915	SL6001	S. Lauwers	Human, feces (Belgium)
R-927	CCUG 33804	CCUG	Human, septic arthritis (Germany)
R-5758	Strain Bower	M. Yuen	Human, blood (Australia)
R-5759	Strain Flint	M. Yuen	Human, blood (Australia)
LMG 8559	CCUG 15432	CCUG	Human, blood (Sweden)
LMG 8558	CCUG 17733	CCUG	Human, feces (Sweden)
LMG 7543^T	CCUG 18818	CCUG	Homosexual man, rectal swab (United States)
LMG 9071	CCUG 18819	CCUG	Homosexual man, blood (United States)
LMG 8770	CCUG 19218	CCUG	Homosexual man, rectal swab (United States)
LMG 9357	CCUG 19503	CCUG	Human, blood (Canada)
LMG 9072	CCUG 19504	CCUG	Human, blood (Canada)
LMG 9153	CCUG 20698	CCUG	Human, feces (United Kingdom)
LMG 16312	CCUG 33887	CCUG	Dog, feces (Sweden)
LMG 13991	M2-08-4658	H. Goossens	Human, feces (Belgium)

[a] Own isolate if not specified.

and the sequences of strains belonging to the same phylogenetic group (retrieved from the EMBL data library) were aligned, and a phylogenetic tree was constructed based on the neighbor-joining method by using the GeneCompar version 2.0 software package (Applied Maths).

The strain numbers and GenBank accession numbers of the strains of the reference species used in the phylogenetic analysis are as follows: "*F. rappini*", CCUG 23435 and M88138; *Helicobacter acinonychis*, LMG 12684^T and M88148; *Helicobacter bilis*, LMG 18386^T and U18766; *Helicobacter bizzozeronii*, R-1051^T and Y09404; *Helicobacter canis*, LMG 18086^T and L13464; *H. cinaedi*, LMG 7543^T and M88150; *Helicobacter cholecystus*, R-3555^T and U46129; *Helicobacter felis*, LMG 11750^T and M37642; *Helicobacter fennelliae*, LMG 7546^T and M88154; *Helicobacter hepaticus*, LMG 16316^T and U07574; *Helicobacter muridarum*, LMG 13646^T and M80205; *Helicobacter mustelae*, LMG 18044^T and M35048; *Helicobacter nemestrinae*, LMG 14378^T and X67854; *Helicobacter pametensis*, LMG 12678^T and M88147; *Helicobacter pullorum*, LMG 16317^T and L36141; *Helicobacter pylori*, LMG 7539^T and M88157; *Helicobacter rodentium*, ATCC 700285^T and U96296; *Helicobacter salomonis*, Inkinen^T and U89351; *Helicobacter* sp. strain Mainz, R-927 and X81028; *Helicobacter trogontum*, R-5081^T and U65103; and *Sulfurospirillum* sp. strain CCUG 13942, L14632.

Nucleotide sequence accession numbers. The nucleotide sequence accession numbers for the 16S rDNA sequences of strains R-4792, LMG 16312, and R-927 are AF207737, AF207738, and AF207739, respectively.

RESULTS

Whole-cell protein electrophoresis. All 14 isolates were identified as *H. cinaedi* by comparison of their whole-cell protein profiles with a database comprising patterns of over 1,000 *Campylobacter*, *Arcobacter*, *Helicobacter*, and *Wolinella* strains. Figure 1 shows the result of the numerical comparison of the protein patterns of the 14 isolates, the eight *H. cinaedi* reference strains, and the type or reference strains of other *Helicobacter* species. All 22 *H. cinaedi* strains form a single protein electrophoretic cluster above a similarity level of 80%.

Phenotypic analysis. Computer-assisted comparison of the biochemical profiles identified 14 of the 15 strains examined (the 14 novel isolates and reference strain LMG 9537) as *H. cinaedi* with a Willcox probability identification score (IDS) of 0.99 (i.e., 99% probable) or more. Field strain LMG 16312 was not confidently identified by this method (IDS, <0.95). Several atypical features (notably results in nitrate reduction, alkaline phosphatase production, indoxyl acetate hydrolysis, tolerance and reduction of triphenyl tetrazolium chloride, growth at 42°C, and resistance to cephalothin and cefoperazone) were noted in the phenotype of LMG 16312 compared with those of type and reference strains determined previously (23). Table 2 summarizes the key phenotypic features of the *H. cinaedi* strains examined here and previously (23) that are especially

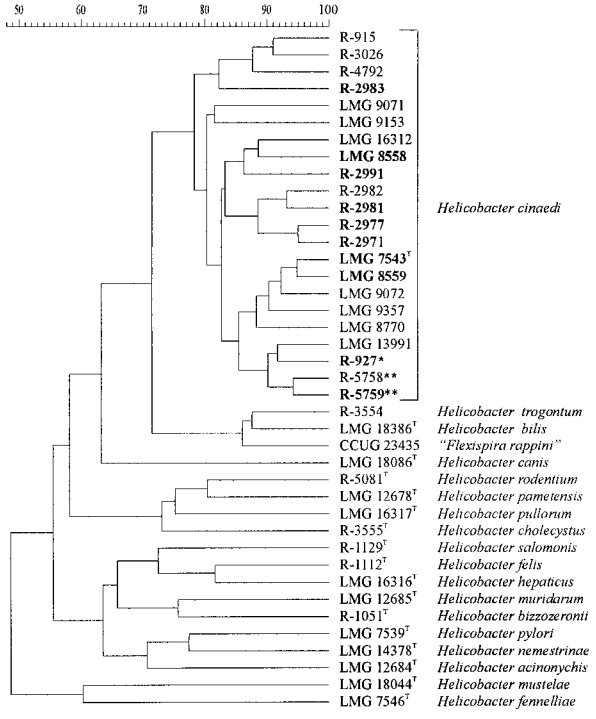

FIG. 1. Dendrogram expressing similarity of whole-cell protein patterns of all 22 *H. cinaedi* isolates and of *Helicobacter* reference strains. The strain numbers in boldface indicate those strains that were included in DNA-DNA hybridization experiments. The strain marked with an asterisk was originally described as *Helicobacter* sp. strain Mainz; the strains marked with double asterisks were originally described as "*H. westmeadii*".

TABLE 2. Selected phenotypic test results of the 15 field and reference strains of *H. cinaedi* examined in the present study compared with data obtained previously for other human enteric *Helicobacter* species (23)

Test	No. of strains giving positive result					
	H. cinaedi		*H. canis* ($n = 11$)	*H. fennelliae* ($n = 12$)	*H. pullorum* ($n = 16$)	"*F. rappini*" ($n = 3$)[a]
	Present study ($n = 15$)	Previous study ($n = 12$)				
Catalase	7	10	0	11	14	1
Urease	0	0	0	0	0	3
Nitrate reduction	11	12	0	0	16	2
Indoxyl acetate hydrolysis	1	1	11	12	0	1
Cephalothin	14	12	2	1	16	3
Carbenicillin	0	1	1	0	14	0
Methyl orange	8	12	0	5	16	1
Sodium fluoride	1	0	0	7	16	0

[a] Data include unpublished results of S. L. W. On for two strains of canine origin.

useful for discriminating among other enteric helicobacters associated with human gastroenteritis or septicemia.

Dot blot DNA-DNA hybridizations. Dot blot DNA-DNA hybridization experiments revealed strong hybridization reactions between the *H. cinaedi* LMG 7543^T probe and all of the *H. cinaedi* strains tested. Weak or no hybridization was obtained to "*F. rappini*" or *H. canis* DNA (the nearest phylogenetic neighbors of *H. cinaedi*) or to any other *Helicobacter* species (Fig. 2).

16S rDNA sequencing and phylogenetic analysis. The expected (~1,500-bp) amplicon was obtained from both R-4792 and R-927, whereas strain LMG 16312 yielded an enlarged amplicon of ~1,700 bp, later found to be due to the presence of an intervening sequence (IVS) of ~200 bp that started at base 168 of the submitted sequence (AF207738) for this strain. The 16S rDNA sequence similarity levels between the different *H. cinaedi* isolates varied considerably. Figure 3 summarizes the 62 polymorphic sites (4.3%) identified after alignment of our *H. cinaedi* sequences with an existing sequence for strain R-927 (accession number X81028) and the 1,444-bp sequence of the *H. cinaedi* type strain (accession number M88150). The majority of these polymorphic sites were accounted for by the two sequences representing strain R-927. The positions where alignment gaps were introduced into each sequence are also shown in Fig. 3. Three of these seven alignment gaps occurred at the two termini of the IVS for strain LMG 16312 (the IVS sequence itself is not shown), and a single-base deletion was also found in this region for strain R-927 (both sequences). The remaining alignment gaps seem likely to be errors in downloaded EMBL sequences, since the 1-base deletion (position 184) and 2-base insertion (position 826) were not found in any of our submitted sequences. The repeat sequence for R-927 was highly similar (99.9%) to the sequence originally described (13), except that a 2-base insertion (detailed above) was seen in the original sequence and a further four polymorphisms were identified (positions 928 and 1419 to 1421 [Fig. 3]), possibly as a result of nucleotide misincorporation during sequencing procedures in either of the two investigations.

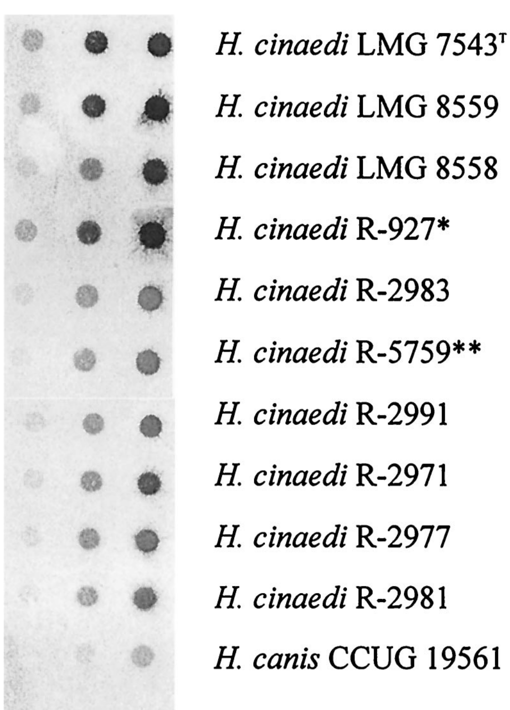

FIG. 2. Dot blot DNA-DNA hybridization result obtained with the labeled *H. cinaedi* LMG 7543^T probe. The strain marked with an asterisk was originally described as *Helicobacter* sp. strain Mainz; the strain marked with double asterisks was originally described as "*H. westmeadii*".

Figure 4 shows the result of neighbor-joining cluster analysis of *H. cinaedi* strains and related bacteria, which was based upon a comparison of 1,375 nucleotides of the 16S rRNA gene. Similarity values between the sequences of the *H. cinaedi* type strain (accession no. M88150), strain R-5759 ("*H. westmeadii*", accession no. U44756; identical 16S rDNA sequences were reported for strains R-5758 and R-5759), strain R-4792, and strain LMG 16312 varied between 99.7 and 98.5%. The levels of 16S rDNA sequence similarity of these *H. cinaedi* isolates to

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                                                                  1111111111111111111
                 1111111111222222445555555555566666677888999999911122222222223333444
              779123334899900012925344555777880000191222722268890350112234551133122
              570870674478908943170719012035355678777267367891820298171859467812901
M88150        TTATTGTTG-GTTTAACTTATTTTAGTCGATCACTAAGGA--CGTACTTCCTCATATTATTTCATGA?C
LMG 16312     .C.......C.---...C.T.C............C....G--.AG...C.T......CG...TGCA.C.
R-4792        .C.......C...........C..G.............G--.AG...C.T......CG...TGCA.C.
R-927         C.GCAACAAC-...TGT.ATA.CATAGGAGCTCTATGAT.--TTGCTCCTTCTGCGA..CCC.....C.
X81028        C.GCAACAA--...TGT.ATA.CATAGGAGCTCTATGAT.ACTTG.TCCTTCTGCGA..CCC....TTA
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FIG. 3. Comparison of *H. cinaedi* 16S rRNA gene sequences with the sequence for the type strain (M88150), showing only polymorphic sites and those sites where gaps (−) were introduced during alignment. X81028 represents a previously published sequence for strain R-927 (13). The numbers (in vertical format) above the sequences correspond to the nucleotide numbering of the M88150 sequence, including three alignment gaps. Bases identical to the M88150 sequence are represented by periods.

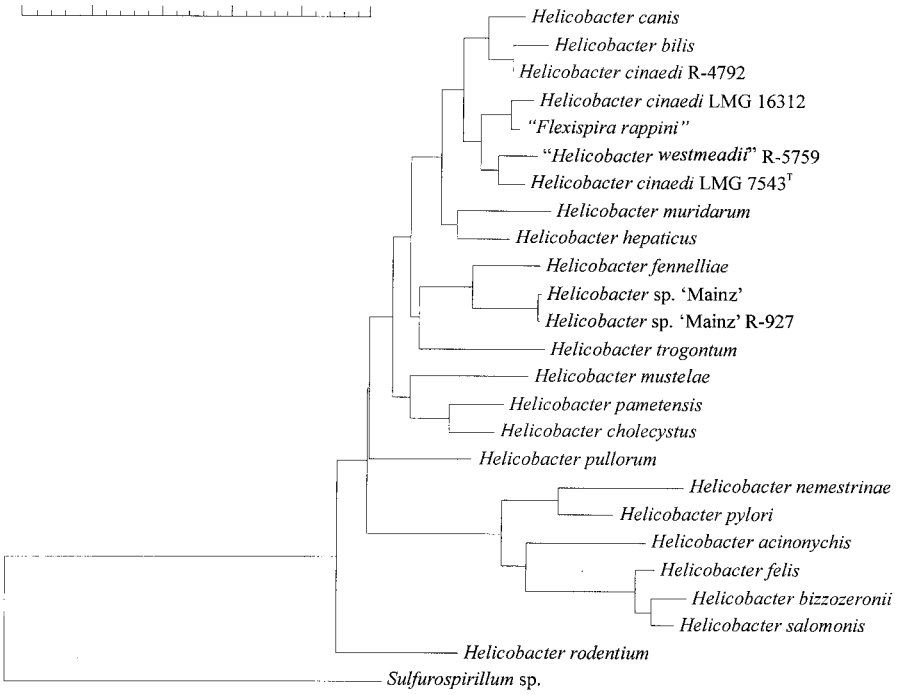

FIG. 4. Neighbor-joining phylogenetic tree of *H. cinaedi* strains and related bacteria based on 16S rRNA sequence comparisons. The scale bar indicates 5% sequence dissimilarity.

the "*F. rappini*" (accession no. M88138) reference strain were in the same range of 99.5 to 98.3%, and similarities to the *H. canis* (accession no. L13464) and *H. bilis* (accession no. U18766) reference strains were only slightly lower (between 97.9 and 98.7%). Not surprisingly, the *H. cinaedi* strains do not form a distinct cluster in the dendrogram but cluster together with the "*F. rappini*," *H. canis*, and *H. bilis* reference strains (Fig. 4). The levels of 16S rDNA sequence similarity of strain R-927 (both sequences) to the other *H. cinaedi* isolates examined were between 97.1 and 96.5% only. The two sequences for this strain were most similar (98%) to that of the *H. fennelliae* reference strain (accession no. M88154), thereby confirming the observations reported by Husmann et al. (13).

DISCUSSION

It is well known and widely accepted that the biochemical identification of *Helicobacter* strains by means of a limited number of commonly used tests is extremely difficult. In the present study, we used two conceptually different approaches to identify 14 putative helicobacters to the species level. The accuracy of the identifications suggested by these methods was checked by DNA-DNA hybridization, which is generally considered the standard for species discrimination (34). Previous studies with a range of different bacteria have demonstrated a correlation between the degree of whole-cell protein pattern similarity as revealed by one-dimensional sodium dodecyl sulfate-PAGE and the level of DNA-DNA hybridization. Vandamme et al. (31) and Costas et al. (2) previously demonstrated that protein pattern analysis is most useful to distinguish *Helicobacter* species. This was recently confirmed by Jalava et al. (15), who compared DNA-DNA hybridization, whole-cell protein electrophoresis, and biochemical analysis for the differentiation of several gastric *Helicobacter* species. Similarly, the application of biochemical examination by means of a panel of 64 tests for the identification of *Helicobacter* strains was validated by the examination of a large collection of well-characterized strains (1, 23).

In the present study, whole-cell protein pattern analysis identified all 14 field isolates unambiguously as *H. cinaedi*, while the probabilistic analysis of over 60 biochemical test results successfully identified 13 of these strains, as well as a reference strain not examined before. A qualitative DNA-DNA hybridization assay of a selection of 10 strains confirmed that all of the strains belonged to a single species. Previous comparative studies of this qualitative DNA-DNA hybridization assay and the quantitative optical renaturation rates method (5) revealed that the former procedure allowed correct species level classification of strains of various *Helicobacter* species (14).

In contrast, identification of several of these isolates based on a restricted set of biochemical tests and their levels of 16S rRNA sequence similarity to known *Helicobacter* species was not straightforward. Husmann et al. (13) reported that strain R-927 represented a novel *Helicobacter* species referred to as *Helicobacter* sp. strain Mainz. In our study, its biochemical reactivity pattern and whole-cell protein profile conformed to those of the other *H. cinaedi* strains (Fig. 1), and DNA-DNA hybridization to the type strain of *H. cinaedi* revealed a very strong signal (Fig. 2). The main reason for the misidentification of this strain was an overreliance on its unique position in the 16S rDNA sequence-based phylogenetic tree (13) (Fig. 4). This strain indeed has a 16S rRNA gene sequence that is strikingly different from those of other *H. cinaedi* strains and is in fact more similar to that of the *H. fennelliae* type strain (Fig. 4). It must be emphasized here that divergence in 16S rRNA gene sequences of up to 4.3% have been reported in another bacterium belonging to the epsilon subdivision of the division *Proteobacteria*, *Campylobacter hyointestinalis* (11). For that species, too, DNA-DNA hybridizations confirmed that these

strains represented a single genomic species as defined in taxonomic practice (34). For species like this, it is not surprising to find that not all strains cluster together in a phylogenetic tree (Fig. 4).

The classification of strains R-5758 and R-5759 as a novel *Helicobacter* species, "*H. westmeadii*" (30), was probably primarily due to failure to grow the isolates under optimal conditions, leading to at least a few equivocal biochemical test results. "*H. westmeadii*" was reported to be an anaerobic helicobacter, thereby differentiating it from *H. cinaedi*, its nearest phylogenetic neighbor (Fig. 4). However, like many other helicobacters, both isolates indeed grew very poorly in a microaerobic environment without hydrogen but grew abundantly when hydrogen was supplemented. Incubation of the strains in an incubator routinely used to work with strict anaerobes (atmospheric composition, 5% CO_2, 10% H_2, and 85% N_2) did not yield visible growth. Further biochemical tests performed under these optimal conditions failed to reproduce hippurate activity, and the overall reactivity pattern corresponded to that of typical *H. cinaedi* strains. Again, DNA-DNA hybridization to the type strain of *H. cinaedi* revealed a very strong signal (Fig. 2), confirming the identification of these isolates as *H. cinaedi*. Therefore, the combined evidence from biochemical, protein electrophoretic, and DNA-DNA hybridization analyses indicate unambiguously that "*H. westmeadii*" is a junior synonym of *H. cinaedi*.

Undoubtedly, comparison of (nearly) entire 16S rDNA sequences is one of the most powerful tools for establishing the phylogenetic neighborhood of an unknown organism. However, many taxonomic studies have revealed that this approach is often not sensitive enough to identify strains to the species level. Indeed, strains belonging to different species may have identical 16S rRNA gene sequences, and strains of one species may have 16S rRNA genes that differ by up to 3% (27) and even over 4% (11) of the total 16S rRNA gene sequence. There is clearly a lack of knowledge, not only of the strain-to-strain variation within a species, but also of the interoperon variation within a single strain. Therefore, concluding that an unidentified isolate belongs to a particular *Helicobacter* species because it shares a high percentage of its 16S rRNA gene sequence or concluding that it represents a novel species because it occupies a unique position in the phylogenetic tree or because it shares only 97% of its 16S rRNA gene sequence with its closest neighbor is premature in the absence of appropriate complementary data.

The identification of the present collection of strains as *H. cinaedi* expands the number of potential reservoirs for infection. *H. cinaedi* was first described by Totten et al. (29) to delineate a group of *Campylobacter*-like organisms isolated from homosexual men suffering from enteritis, proctitis, or proctocolitis and has subsequently been isolated in cases of meningitis, bacteremia, and enteritis in humans, mainly those with immature or compromised immune systems (24, 31, 32). Recently, Weir et al. (35) described another helicobacter isolated from the blood of a patient with AIDS. All available information suggests that this isolate is *H. cinaedi* too. The whole-cell fatty acid and biochemical profiles conform with those of typical *H. cinaedi* strains (note that Trivett-Moore et al. [30] reported the "*H. westmeadii*" strains to be indistinguishable from *H. cinaedi* when examined by means of cellular fatty acid analysis). Indeed, the authors reported the absence of nitrate reduction as a criterion to separate their strain from *H. cinaedi*, but of a total of 26 *H. cinaedi* strains present in our biochemical database (data from the present study and from reference 2), 4 did not reduce nitrate. Absence of nitrate reduction is clearly not uncommon in *H. cinaedi*, and as discussed above, from the perspective of species level identification, the reliability of using 16S rDNA sequence similarity levels between 98.7 and 99.2% (35) is suspect, since intraspecies variation greater than this has been reported on more than one occasion. Our growing knowledge on the multiple reservoirs of *H. cinaedi* supports the hypothesis of Weir and coworkers that *H. cinaedi* (including "*H. westmeadii*" and *Helicobacter* sp. strain Mainz) may represent a *Helicobacter* species that is prone to cause sepsis in immunocompromised patients, such as those with AIDS. We now know that this bacterium occurs in hamsters (8), cats and dogs (16) (Table 1), and foxes and rats (Table 1). Its role in these animal hosts is not known. Good incubation and identification strategies will be required to establish the genuine significance of this *Helicobacter* species in bacteremic and enteric disease. Identification strategies by means of whole-cell protein or fatty acid analysis, extended biochemical testing, or restriction profile analysis of PCR amplicons derived from the 23S rRNA gene (12, 17) should be considered to support tentative identification results obtained by comparison of complete 16S rRNA genes. As useful as the latter method is, present data clearly indicate that it cannot be regarded as the "gold standard" for species-level identification of many members of the epsilon subdivision of the division *Proteobacteria* (*Helicobacter*, *Campylobacter*, *Arcobacter*, and related bacteria).

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