NOTE

Helicobacter nemestrinae* ATCC 49396^T is a strain of *Helicobacter pylori* (Marshall *et al.* 1985) Goodwin *et al.* 1989, and *Helicobacter nemestrinae* Bronsdon *et al.* 1991 is therefore a junior heterotypic synonym of *Helicobacter pylori

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***Helicobacter nemestrinae* Bronsdon *et al.* 1991, a gastric helicobacter species isolated from a pigtailed macaque, is thought to be the species most closely related to the important human pathogen *Helicobacter pylori*. The only available strain of this taxon is the type strain, ATCC 49396^T. We sequenced seven housekeeping genes and two flagellin genes for *H. nemestrinae* ATCC 49396^T. If ATCC 49396^T were a separate species, these sequences should have been distinct from those of *H. pylori*. Instead, all sequences clustered together with sequences obtained previously for 20 or more *H. pylori* isolates from diverse geographical locations. The 16S rDNA sequence differed from that reported previously for this strain by 38 nucleotides and was most similar to that of *H. pylori* 85D08 (accession no. U00769), which was isolated from a rhesus macaque. It differed by less than 1% from 16S rDNA sequences of numerous other *H. pylori* strains, including the type strain, NCTC 11637^T (= ATCC 43504^T). These data indicate that the strain currently distributed as *H. nemestrinae* ATCC 49396^T is really a strain of *H. pylori* and that *H. nemestrinae* Bronsdon *et al.* 1991 is a junior heterotypic synonym of *Helicobacter pylori* (Marshall *et al.* 1985) Goodwin *et al.* 1989.**

Keywords: *Helicobacter pylori*, *Helicobacter nemestrinae*, taxonomy, multilocus sequence analysis, 16S rDNA sequencing

The gastric mucus of many different animal species is colonized by gastric *Helicobacter* species. The best-studied of these is *Helicobacter pylori*, a prevalent human pathogen that colonizes the gastric mucus of more than half of the human population and causes chronic, often lifelong infections. *Helicobacter nemestrinae* T81213-NTB^T (= ATCC 49396^T) was isolated from a pigtailed macaque (*Macaca nemestrina*) and has been reported to be closely related to *H. pylori* (Bronsdon *et al.*, 1991). *H. nemestrinae* was established as a separate species, with ATCC 49396^T as the type

strain, because the G + C content was much lower than that of *H. pylori* (24 versus 35–41 mol%) and because there was less than 10% DNA–DNA relatedness between ATCC 49396^T and *H. pylori*. The species designation of *H. nemestrinae* was confirmed by 16S rDNA sequence comparisons, which also showed it to be distinct from *H. pylori* (Sly *et al.*, 1993). To our knowledge, ATCC 49396^T is the only existing strain of *H. nemestrinae*.

H. nemestrinae is currently regarded as the *Helicobacter* species most closely related to *H. pylori*, followed by *Helicobacter acinonychis* (originally named '*Helicobacter acinonyx*'), a gastric spiral organism that has been isolated repeatedly from cheetahs (Eaton *et al.*,

The GenBank/EMBL/DBJ accession numbers for the sequences reported in this paper are AJ421784–AJ421792.

Fig. 1. Selected trees (neighbour-joining trees of Jukes–Cantor-corrected pairwise distances) showing the relationship between nucleotide sequences from *H. nemestrinae* ATCC 49396^T and strains of *H. pylori* from diverse geographical locations as well as other *Helicobacter* species (sequences for other *Helicobacter* species were only available for *flaA* and *flaB* flagellin genes). Trees for *flaA* (a), *flaB* (b), *efp* (c) and *atpA* (d) are shown. hf *flaA*, *H. felis flA*; hm *flaA*, *H. mustelae flA*; hf *flaB*, *H. felis flB*; hm *flaB*, *H. mustelae flB*.

1993). As part of a larger multilocus sequence study of population structure and evolution of *H. pylori*, we investigated the related species *H. nemestrinae*.

H. nemestrinae ATCC 49396^T was obtained directly from the ATCC and grown on blood-agar plates for 2 d in an atmosphere containing 5% CO₂, 5% H₂ and 90% N₂ in an anaerobic jar at 37 °C. DNA was extracted by the QiaAmp Tissue kit (Qiagen) and used to amplify and sequence fragments of nine genes as described previously (Achtman *et al.*, 1999; Suerbaum *et al.*, 1998). The fragments sequenced were from seven housekeeping genes, *atpA*, *efp*, *mutY*, *ppa*, *trpC*, *ureI* and *yphC*, as well as the two flagellin genes *flaA* and *flaB*. Details about the fragments and the primers used for sequencing have been provided elsewhere (Achtman *et al.*, 1999; Suerbaum *et al.*, 1998). All fragments could be amplified from *H. nemestrinae* DNA and sequencing was performed on both strands. The seven

housekeeping gene fragments were aligned with sequences from 20 strains of *H. pylori* from diverse geographical sources (Achtman *et al.*, 1999). The flagellin gene sequences were aligned with sets of sequences from 54 German (*flaB*) or 33 Canadian (*flaA*) strains as well as 22 South African strains (*flaA* and *flaB*) (Suerbaum *et al.*, 1998). For the two flagellin genes, the corresponding sequences from *Helicobacter mustelae* and *Helicobacter felis* (Josenhans *et al.*, 1995) were included in the tree (Josenhans *et al.*, 1999).

With one exception, the *H. nemestrinae* sequences differed from all other *H. pylori* sequences obtained previously in the laboratory in the course of extensive previous comparative sequencing studies for multiple *H. pylori* strains. However, surprisingly, all nine sequences obtained from *H. nemestrinae* ATCC 49396^T clustered within the *H. pylori* sequences, whereas the flagellin sequences of *H. mustelae* and *H.*

felis were widely separated from the *H. pylori* sequences. Four representative examples of the trees obtained are shown in Fig. 1. The G + C content of the nine *H. nemestrinae* fragments was between 38.6 and 49.6 mol%, widely different from the 24 mol% reported for the whole chromosome of *H. nemestrinae*. The difference between the G + C contents of the *H. nemestrinae* fragments and the mean G + C contents calculated for the corresponding *H. pylori* sequences (Achtman *et al.*, 1999) was less than 1.6 mol% for *yphC* and less than 1 mol% for all other fragments. All these results were unexpected if *H. nemestrinae* ATCC 49396^T was a strain of a species separate from *H. pylori*.

We subsequently obtained a 16S rDNA sequence for *H. nemestrinae* strain ATCC 49396^T (accession no. AF348617). The sequence differed by 38 out of 1460 aligned nucleotides from the published sequence of *H. nemestrinae* ATCC 49396^T (accession no. X67854). In addition to these differences, the published sequence differed by one 4-bp deletion and two 1-bp deletions from the sequence obtained by us. Of all 16S rDNA sequences in the public databases, our sequence was most similar (four differences out of 1477 aligned nucleotides) to the sequence of *H. pylori* strain 85D08 (accession no. HPU00679), an *H. pylori* strain that was isolated from a rhesus macaque in the USA (Drazek *et al.*, 1994). It was over 99% identical to numerous other 16S rDNA sequences of *H. pylori* strains, including the two strains whose genome sequences are available, 26695 and J99, and the type strain, ATCC 43504^T (= NCTC 11637^T). Interestingly, the ATCC 49396^T sequence was also identical to a (partial) 16S rDNA sequence from a rhesus macaque isolate obtained at the German Primate Centre in Göttingen (data not shown).

We conclude that the strain currently distributed as the type strain of *H. nemestrinae*, ATCC 49396^T, differs from the previous description of this strain and is, with very high probability, a strain of *H. pylori*. We did not perform DNA–DNA hybridizations or fatty acid analyses on ATCC 49396^T, but it has been reported that other investigators have made similar observations about ATCC 49396^T using phenotypic methods (On, 2000).

At present, the reason for the discrepancy between our data and the data published by Bronsdon *et al.* (1991) and Sly *et al.* (1993) is unclear. The 16S rDNA sequencing results were reproduced with a sample of ATCC 49396^T grown from a ‘seed vial’ that was kindly provided by the ATCC and had been produced

in the initial deposition process in 1990. According to ATCC records, one vial from this batch had been controlled and approved by the depositors. Despite the co-operation of three authors of the earlier studies (E. Stackebrandt, L. Sly and M. Bronsdon), it has not yet been possible to obtain an isolate of *H. nemestrinae* ATCC 49396^T (or T81213-NTB^T) that fits the original description.

Consequently, we propose that *H. nemestrinae* ATCC 49396^T should be recognized as a strain of *H. pylori* and that *Helicobacter nemestrinae* Bronsdon *et al.* 1991 is therefore not a separate species, but a junior heterotypic synonym of *Helicobacter pylori* (Marshall *et al.* 1985) Goodwin *et al.* 1989.

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