**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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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 acinyx'), a gastric spiral organism that has been isolated repeatedly from cheetahs (Eaton et al.,...
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 49396T was obtained directly from the ATCC and grown on blood-agar plates for 2 d in an atmosphere containing 5% CO<sub>2</sub>, 5% H<sub>2</sub> and 90% N<sub>2</sub> 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 49396T 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 mol% for all other fragments.

We subsequently obtained a 16S rDNA sequence for *H. nemestrinae* strain ATCC 49396T (accession no. AF348617). The sequence differed by 38 out of 1460 aligned nucleotides from the published sequence of *H. nemestrinae* ATCC 49396T (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 43504T (= NCTC 11637T). Interestingly, the ATCC 49396T 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 49396T, 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 49396T, but it has been reported that other investigators have made similar observations about ATCC 49396T 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 49396T 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 49396T (or T81213-NTB2) that fits the original description.

Consequently, we propose that *H. nemestrinae* ATCC 49396T 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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### References


