NOTE

**Helicobacter nemestrinae ATCC 49396** 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 49396T. We sequenced seven housekeeping genes and two flagellin genes for H. nemestrinae ATCC 49396T. If ATCC 49396T 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 11637T (= ATCC 43504T). These data indicate that the strain currently distributed as H. nemestrinae ATCC 49396T 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 (= ATCC 49396T) was isolated from a pigtailed macaque (Macaca nemestrina) and has been reported to be closely related to H. pylori (Bromsdon et al., 1991). H. nemestrinae was established as a separate species, with ATCC 49396T 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 49396T 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 49396T 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., 1991). The GenBank/EMBL/DDBJ 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<sup>T</sup> 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* *flaA*; hm *flaA*, *H. mustelae* *flaA*; hf *flaB*, *H. felis* *flaB*; hm *flaB*, *H. mustelae* *flaB*.

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<sup>T</sup> 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 <i>et al.</i>, 1999; Suerbaum <i>et al.</i>, 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 <i>et al.</i>, 1999; Suerbaum <i>et al.</i>, 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 <i>et al.</i>, 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 <i>et al.</i>, 1998). For the two flagellin genes, the corresponding sequences from *Helicobacter mustelae* and *Helicobacter felis* (Josenhans <i>et al.</i>, 1995) were included in the tree (Josenhans <i>et al.</i>, 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<sup>T</sup> clustered within the *H. pylori* sequences, whereas the flagellin sequences of *H. mustelae* and *H.
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We subsequently obtained a 16S rDNA sequence for *H. nemestrinae* strain ATCC 49396<sup>T</sup> (accession no. AF348617). The sequence differed by 38 out of 1460 aligned nucleotides from the published sequence of *H. nemestrinae* ATCC 49396<sup>T</sup> (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 (Drazeck 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<sup>T</sup> (= NCTC 11637<sup>T</sup>). Interestingly, the ATCC 49396<sup>T</sup> 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<sup>T</sup>, 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<sup>T</sup>, but it has been reported that other investigators have made similar observations about ATCC 49396<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> (or T81213-NTB<sup>T</sup>) that fits the original description.

Consequently, we propose that *H. nemestrinae* ATCC 49396<sup>T</sup> 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.

### Acknowledgements

We thank Mark Achtman and one anonymous reviewer for reading the manuscript and helpful suggestions for its improvement. We thank Melinda Bronsdon and Don Bark for supplying early stock cultures of T81213-NTB<sup>T</sup>/ATCC 49396<sup>T</sup>. This work was supported by grant SU 133/3-2 from the Deutsche Forschungsgemeinschaft to S.S.

### References


