Helicobacter heilmannii sp. nov., isolated from feline gastric mucosa

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Three Gram-negative, microaerophilic bacteria with a corkscrew-like morphology isolated from the gastric mucosa of cats and designated ASB1^T, ASB2 and ASB3, were subjected to a polyphasic taxonomic study. The isolates grew on biphasic culture plates in microaerobic conditions at 37°C and exhibited urease, oxidase and catalase activity. They were also able to grow in colonies on dry agar plates. Based on 16S rRNA gene sequence analysis, ASB1^T, ASB2 and ASB3 were identified as members of the genus *Helicobacter* and showed 98 to 99% sequence similarity to *H. felis*, *H. bizzozeronii*, "*Candidatus* H. heilmannii", *H. cynogastricus*, *H. baculiformis* and *H. salomonis*, six related *Helicobacter* species previously detected in the feline or canine gastric mucosa. Sequencing of the partial *hsp60* gene demonstrated that ASB1^T, ASB2 and ASB3 constitute a separate taxon among the feline and canine *Helicobacter* spp. The urease gene sequences of ASB1^T, ASB2 and ASB3 showed approximately 91% similarity to the urease gene sequences of "*Candidatus* Helicobacter heilmannii". Protein profiling, the absence of alkaline phosphatase activity and several other biochemical characteristics also allowed to differentiate the strains ASB1^T, ASB2 and ASB3 from other *Helicobacter* species of feline or canine gastric origin. The results of this polyphasic taxonomic study show that the cultured isolates constitute a new taxon corresponding to "*Candidatus* Helicobacter heilmannii" previously demonstrated in the stomach of humans, wild felidae, cats and dogs. The name *Helicobacter heilmannii* sp. nov. is proposed for these new isolates.

Long, spiral-shaped bacteria belonging to the genus *Helicobacter* have been demonstrated in the gastric mucosa of man and several animal species (Haesebrouck *et al.*, 2009). *Helicobacter* (*H.*) *pylori* is the most common and best known gastric *Helicobacter* species in humans. Today, a large number of gastric non-*Helicobacter pylori Helicobacter* spp., provisionally named *H. heilmannii* and naturally colonizing the stomach of animals, have also been described in humans. Sequence analysis of 16S rRNA genes detected in '*Helicobacter heilmannii*'-positive gastric biopsies revealed the presence of two sequence types. This has led to the sub classification of the non-*Helicobacter pylori* helicobacters into '*Helicobacter heilmannii*' type 1 and '*Helicobacter heilmannii*' type 2. *H. heilmannii* type 1 represents a single *Helicobacter* species, namely *H. suis*. '*H. heilmannii*' type 2 represents a group of species, including *H. felis*, *H. bizzozeronii*, *H. salomonis*, and '*Candidatus* H. heilmannii'(Baele *et al.*, 2008a; Haesebrouck *et al.*, 2009).

The first *Helicobacter* species isolated from the stomach of cats and dogs was *H. felis* (Lee *et al.*, 1988). Later on, *H. bizzozeronii, H. salomonis, H. baculiformis* and *H. cynogastricus* were also isolated from the feline and canine gastric mucosa (Baele *et al.*, 2008b; Hänninen *et al.*, 1996; Happonen *et al.*, 1996; Jalava *et al.*, 1998; Jalava *et al.*, 2001; Van den Bulck *et al.*, 2006). *H. cynogastricus* and *H. baculiformis* have not yet been detected in the human gastric mucosa. A sixth long spiral shaped *Helicobacter* sp. has been detected in wild feline and human, as well as in canine and feline gastric biopsies (Neiger *et al.*, 1998; Hwang *et al.*, 2002; O'Rourke *et al.*, 2004b). It could not be cultured *in vitro* and was provisionally named '*Candidatus* Helicobacter heilmannii' (O' Rourke *et al.*, 2004b). Based on 16S rRNA gene sequence analysis, all these species are phylogenetically highly related to each other (Solnick *et al.*, 1993). The similarity of their *ureAB* urease genes is, however, lower than 85%, allowing discrimination between these species (O'Rourke *et al.*, 2004b). The uncultured "*Candidatus* Helicobacter heilmannii" was found with a prevalence ranging from 20% to

100% in the gastric mucosa of both cats and dogs (Haesebrouck *et al.*, 2009; Hwang *et al.*, 2002; Neiger *et al.*, 1998; Van den Bulck *et al.*, 2005). It was detected in 8-19% of gastric biopsy samples of humans with histological evidence of a non-*Helicobacter pylori Helicobacter* infection (Haesebrouck *et al.*, 2009; Trebesius *et al.*, 2001; Van den Bulck *et al.*, 2005). Moreover, "*Candidatus* Helicobacter heilmannii" has been propagated in mice for up to 28 months and was able to induce mucosa associated lymphoid tissue (MALT) lymphomas in the stomach of these animals (O'Rourke *et al.*, 2004a).

In this study, we describe the successful isolation of "*Candidatus* Helicobacter heilmannii" *in vitro* and the characterisation of this species using a polyphasic taxonomic study.

The strain, designated ASB1^T (= type strain), was isolated from the mucosa of the stomach of a cat euthanized at a shelter for homeless cats, Sint-Niklaas, Belgium. The two other strains, designated ASB2 and ASB3, were isolated from the mucosa of the stomach of cats (positive for feline immunodeficiency virus) euthanized at the faculty of Veterinary Medicine, Ghent University, Belgium.

The stomachs of these 3 cats were submersed in a 1% HCl bath for 1 hour (Gruntar *et al.*, 2003). The mucus was scraped off using a glass slide, and collected in a sterile tube.

It was inoculated on *Brucella* agar plates supplemented with 20% (v/v) foetal calf serum, 5 mg/l amphotericin B (Fungizone; Brystal-Myers Squibb, New York, USA), *Campylobacter*-selective supplement (Skirrow, Oxoid, Aalst, Belgium; containing 10 mg/l vancomycin, 5 mg/l trimethoprim lactate and 2500 U/l polymyxin B), Vitox supplement (Oxoid), 0.1% activated charcoal and ca. 0.05% HCl to obtain a pH of 5. The mucus on these agar plates was slightly liquefied with *Brucella* broth containing 20% foetal calf serum. The plates were incubated at 37°C under microaerobic conditions with a gas mixture of 10% CO_2, 5% O_2 and 85% N_2. Plates were checked every day and *Brucella* broth (pH 5) supplemented with 20%

foetal calf serum was added to the agar surface to ensure that the plates did not become dry. Primary growth was examined by light microscopy, revealing the presence of large, spiral-shaped and motile bacterial cells. Growth of subcultures occurred as a spreading layer on moist agar plates. Bacterial cells were harvested in *Brucella* broth and stored at -70°C in a medium consisting of 7.5 g glucose, 25 ml *Brucella* broth and 75 ml sterile inactivated foetal calf serum.

Genomic DNA of isolates ASB1^{T}, ASB2 and ASB3 was extracted using PrepMan sample preparation reagent from Applied Biosystems as described by the manufacturer.

The 16S rRNA gene was amplified using the commercially available Qiagen Taq Mastermix and primers *αβ*-NOT (5'-TCAAACTAGGACCGAGTC-3') and *ω*MB (5'-TACCTTGTTACTTCACCCCA-3') as described by Baele *et al.*, 2001. The PCR products were sequenced using the BigDye Terminator sequencing kit (Applied Biosystems, California, USA) and primers pD, Gamma*, 3 and O* (Coenye *et al.*, 1999). Sequences were determined on an automatic DNA sequencer (ABI Prism 3100 Genetic analyser; Applied Biosystems) and the electropherograms were exported and converted to the VectorNTI software (Invitrogen, Merelbeke, Belgium). The sequences were compared with the NCBI genbank by using the BLAST search tool. All *Helicobacter* species with validly published names (http://www.bacterio.cict.fr/h/helicobacter.html) were included for phylogenetic analysis. Phylogenetic analysis was performed using the ClustalW, BioEdit and Jalview software tools. Multiple alignment was determined using ClustalW with an open gap penalty of 100% and a unit gap penalty of 0%. A phylogenetic tree, with *H. pylori* as outgroup, was constructed using the neighbour-joining method and is shown in Fig. 1. The 16S rRNA gene sequences of strains ASB1^{T}, ASB2 and ASB3 showed more than 98% sequence similarity

with each other (Genbank accession no. HM625820, HM625819, HM625818) and with a sequence from Genbank, Genbank no. AF506786 (originating from '*Candidatus* H. heilmannii' detected in human gastric mucosa, O'Rourke et al., 2004b). The most closely related organisms were '*Candidatus* H. heilmannii', *H. felis*, *H. bizzozeronii*, *H. salomonis* and *H. suis* with a sequence similarity ranging from 93% to 98% with the novel strains.

Sequence analysis of the urease gene has been found to be more discriminatory than the 16S rRNA gene for species differentiation between gastric *Helicobacter* species of animal origin (O'Rourke *et al.*, 2004b). Therefore, the sequences of partial fragments of the *ureA* and *ureB* genes, including a spacer region, were determined after amplification using primers U430F and U1735R (1224 bp amplicon) (O'Rourke *et al.*, 2004b). The sequences were compared with the NCBI genbank by using the BLAST search tool. Based on the phylogenetic tree, reconstructed from genetic distances of the 16r RNA gene sequences, the most closely related *Helicobacter* species were included for phylogenetic analysis of the *ureaAB* gene. Phylogenetic analysis was performed using the same software tools as described for the 16S rRNA gene. A phylogenetic tree, with *H. pylori* as outgroup, was constructed using the neighbour-joining method and is shown in Fig. 2.

Isolates ASB1^{T}, ASB2 and ASB3 showed 98% similarity with each other (Genbank accession no. HM625826, HM625825, HM625824) and 91% similarity with *ureaAB* gene sequences from the '*Candidatus* H. heilmannii' strains, detected in human and wild feline gastric mucosa and previously deposited in Genbank (O'Rourke *et al.*, 2004b). Moreover, these 3 isolates clustered with the '*Candidatus* H. heilmannii' (Fig. 2). The phylogenetic neighbours were the following species: *H. bizzozeronii* (about 84% similarity), *H. suis* (about 82% similarity), *H. felis* (about 76% similarity), *H. cynogastricus* (about 76% similarity) and *H. salomonis* (about 75% similarity).

Mikkonen *et al.* (2004) showed that conserved partial 60 kDa heat-shock protein (HSP60) gene sequences give additional phylogenetic information that is useful for differentiating *Helicobacter* species. The *hsp60* gene sequences of the '*Candidatus* H. heilmannii' strains described by O'Rourke *et al.* (2004b) are not available from Genbank. A 550 bp sequence was obtained for ASB1^T, ASB2 and ASB3 using the methodology as described by Mikkonen *et al.* (2004). The sequences were compared with the NCBI genbank by using the BLAST search tool. Based on the phylogenetic tree, reconstructed from genetic distances of the 16r RNA gene sequences, the most closely related *Helicobacter* species were included for phylogenetic analysis of the *hsp60* gene. Phylogenetic analysis was performed using the same software tools as described for the 16S rRNA gene. A phylogenetic tree, with *H. pylori* as outgroup, was constructed using the neighbour-joining method and is shown in Fig. 3. The partial hsp60 gene sequences of ASB1^T, ASB2 and ASB3 showed approximately 95% sequence similarity with each other (Genbank accession no. HM625823, HM625822, HM625821). Gene sequence similarities of 85-86%, 84-86%, 84%, 84%, 83% and 81% were obtained for *H. felis*, *H. bizzozeronii*, *H. salomonis*, *H. cynogastricus*, *H. baculiformis* and *H. suis*, respectively, yielding sufficient difference to consign isolates ASB1^T, ASB2 and ASB3 into a new taxon.

Polyacrylamide gel electrophoresis (PAGE) of whole cell proteins of strain ASB1^T, ASB2 and ASB3 and of *H. pylori*, *H. bizzozeronii*, *H. felis*, *H. salomonis*, *H. cynogastricus*, *H. baculiformis* and *H. suis* reference strains (Jalava *et al.*, 1998, 2001; Van den Bulck *et al.*, 2006) was performed in order to establish its distinct taxonomic status with other cultured species of the genus *Helicobacter*. For this purpose, strains were grown on *Brucella* agar supplemented with 20% foetal calf serum, 5 mg/l amphotericin B, *Campylobacter*-selective supplement, Vitox supplement and ca. 0.05% HCl to obtain a pH of 5. Plates were incubated

at 37°C in a microaerobic atmosphere as described above. Whole-cell protein extracts were prepared and SDS-PAGE was performed as described previously (Pot *et al.*, 1994). Sodium dodecyl sulphate PAGE and sample preparation were performed using Criterion™ XT 12% (w/v acrylamide) Bis-Tris precast gels with XT MOPS denaturing running buffer according to the manufacturer's instructions (BIO-RAD, Nazareth, Belgium), but without heating the samples before loading. Staining was performed with Bio-Safe™ Coomassie stain, according to the manufacturer's instructions (BIO-RAD).

Visual and numerical analysis of the protein profiles demonstrated that strains ASB1^T, ASB2 and ASB3 can be clearly distinguished from those of their closest phylogenetic neighbours (Fig. S1). These differences are not limited to one or a few bands but are apparent in the entire profile.

The morphology of strain ASB1^T, ASB2 and ASB3 was studied by means of transmission electron microscopy (TEM) as described by Houf *et al.* (2005) and Mast *et al.* (2005) (Fig. 4). Isolates ASB1^T, ASB2 and ASB3 presented tightly coiled spiral-shaped cells with up to nine turns, that were approximately 3 to 6.5 µm long and approximately 0.6 to 0.7 µm wide (Fig. 4). The length and width were variable depending on the state of contraction. No periplasmic fibrils were observed and coccoid cells predominated in older cultures. Up to 10 sheathed blunt-ended flagella were found at both ends.

According to the recommendations of Dewhirst *et al.* (2000), biochemical and tolerance tests were carried out. Growth of strain ASB1^T, ASB2 and ASB3 was determined on *Brucella* agar plates supplemented with 20% foetal calf serum, 5 mg/l amphotericin B, *Campylobacter*-selective supplement, Vitox supplement and 0.05% HCl to obtain a pH of 5 at 25, 37 and 42°C under microaerobic conditions and at 37°C under aerobic, anaerobic and microaerobic conditions. Tolerance to 1% bile, 1% glycine and 1.5% NaCl was determined on Brucella

agar plates with the same supplements as described above. Growth was also studied on BHI agar, Brucella agar and Mueller-Hinton agar (Oxoid), supplemented with 20% foetal calf serum or 10% defibrinated horse blood, Vitox and Skirrow supplements, amphotericin B and HCl to pH of 5. The plates were incubated for several days in a microaerobic atmosphere at 37°C. Cells are also able to grow in colonies on dry agar plates.

The isolates were also examined for catalase activity by adding a 3% H_2O_2 solution and observing the reaction within 5s. Oxidase activity was performed with Bactident Oxidase strips (Merck, Overijse, Belgium). Following characteristics were studied using the API Campy identification system (BioMérieux, Marc L'Etoile, France): urease activity, reduction of nitrate, esterase activity, hydrolysis of hippurate, γ-glutamyltransferase activity, reduction of triphenyl-tetrazoliumchloride (TTC), alkaline phosphatase activity and pyrrolidonyl, L-arginine and L-aspartate arylamidase activity. Tests were read after 24h incubation at 37°C in an aerobic atmosphere.

Growth on Mueller-Hinton II agar plates supplemented with 5 µg/ml metronidazole and 10% horse blood was also established. The results are listed in the species description below and a comparison of the most important phenotypic characteristics of strains ASB1^T, ASB2 and ASB3 with those of other gastric species of the genus *Helicobacter* is shown in Table 1. The absence of alkaline phosphatase activity and several other characteristics allowed to differentiate strains ASB1^T, ASB2 and ASB3 from their closest phylogenetic neighbours.

In conclusion, the phylogenetic analysis of the 16S rRNA, *ureAB* and *hsp60* genes and the whole-cell protein electrophoresis revealed that strains ASB1^T, ASB2 and ASB3 represent a novel species within the phylogenetic lineage that currently consists of *H. felis, H. bizzozeronii, H. salomonis, H. baculiformis, H. cynogastricus* and *H. suis*.

Description of *Helicobacter heilmannii* sp. nov.

Helicobacter heilmannii (heil.mann'i.i. N.L. gen. n. of Heilmann, in honour of Konrad Heilmann who described the first large case study of gastrospirilla infections in humans (Heilmann & Brochard, 1991)).

Cells are tightly coiled spirals with up to nine turns, that are approximately 3 to 6.5 µm long and approximately 0.6 to 0.7 µm wide. No periplasmic fibrils were observed and coccoid cells predominated in older cultures. They are motile by means of tufts of up to 10 sheathed blunt-ended flagellae at both ends of the cells. Cells are Gram-negative and non-sporulating.

Growth is observed on BHI agar, Brucella agar and on Mueller-Hinton agar supplemented with 20% fetal calf serum or with 10% defibrinated horse blood. Cells are also able to grow in colonies on dry agar plates. Grows in micro-aerophilic conditions and weakly growth is seen after anaerobic incubation. Growth is detected at 37°C, but not at 25 or 42°C. No growth on media supplemented with 1% bile, 1.5% NaCl or 1% glycine. Oxidase-, catalase- and urease-positive. Reduces TTC, nitrate and esterase and tests positive for γ-glutamyltransferase, hippurate and L-arginine arylamidase. Activity of pyrrolidonyl arylamidase, L-aspartate arylamidase, indoxyl acetate hydrolysis and alkaline phosphatase was not detected. Its clinical significance in cats is unknown. *H. heilmannii,* as well as other gastric non-*H. pylori* *Helicobacter* species have been associated with gastritis, gastric and duodenal ulcers and low grade MALT lymphoma of the stomach in humans (Haesebrouck *et al.*, 2009). *H. heilmannii* has been shown to induce MALT lymphomas when propagated in mice for up to 28 months (O'Rourke *et al.*, 2004a).

The type strain, ASB1^{T} (DSM 23983, LMG 26292), was isolated from the gastric mucosa of a cat.

Nucleotide sequence accession numbers

The 16S rRNA gene sequences of *H. heilmannii* ASB1^T (= type strain), ASB2 and ASB3 are available from GenBank under accession number HM625820, HM625819 and HM625818, respectively. The partial *ureAB* gene sequences of *H. heilmannii* ASB1^T, ASB2 and ASB3 are available from GenBank under accession number HM625826, HM625825 and HM625824, respectively. The *hsp60* gene sequences of *H. heilmannii* ASB1^T, ASB2 and ASB3 are available from GenBank under accession number HM625823, HM625822 and HM625821, respectively.

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341 **Table 1.** Differential characteristics of strains ASB1^{T}, ASB2 and ASB3 and other species of the genus *Helicobacter*. Urease activity was

342 uniformly present; growth in the presence of 1% glycine was uniformly absent.

Characteristics	***H. heilmannii sp. nov.***	***H. suis*[#]**	***H. baculiformis*[§]**	***H. cynogastricus*[†]**	***H. bizzozeronii*[‡]**	***H.* felis[‡,¥,¶]**	***H. salomonis*[¶]**	***H. pylori*[‡,¶]**
Cell length (µm)	3-6.5	2.3-6.7	10	10-18	5-10	5-7.5	5-7	2.5-5
Cell width (µm)	0.6-0.7	0.9-1.2	1	0.8-1.0	0.3	0.4	0.8-1.2	0.5-1.0
Nitrate reduction	+	-	+	+	+	+	+	-
Alkaline phosphatase activity	-	+	+	+	+	+	+	+
Hydrolysis of indoxyl acetate	-	-	-	-	+	-	+	-
Growth on/at:								
42°C	-	-	-	-	+	-	-	-
Periplasmic fibril	-	-	+	+	-	+	-	-
No. of flagella per cell	4-10	4-10	11	6-12	10-20	14-20	10-23	4-8
Distribution of flagella[*]	BP	BP	BP	BP	BP	BP	BP	MP

343 [*]BP, bipolar; MP, monopolar; [#]Baele *et al.* (2008a); [§]Baele *et al.* (2008b); [†]Van den Bulck *et al.* (2006); [‡]Hänninen *et al.* (1996); [¥]Lee *et al.*

344 (1988); [¶]Jalava *et al.* (1997)

345 **Figure legends**

346 **Fig. 1.** A phylogenetic tree, reconstructed from genetic distances, based on 16S rRNA gene
347 sequences for the *H. heilmannii* sp. and other *Helicobacter* species. The numbers by the
348 branches indicate the number of times out of 100 that the clade was recovered by bootstrap
349 resampling (number of bootstraps: 100).

350

351 **Fig. 2.** A phylogenetic tree, reconstructed from genetic distances, based on the partial *ureA*
352 and *ureB* gene sequences for the *H. heilmannii* sp. and other urease-positive gastric
353 *Helicobacter* species. Bootstrap values are indicated.

354

355 **Fig. 3.** A phylogenetic tree, reconstructed from genetic distances, based on the partial hsp60
356 gene sequences for the *H. heilmannii* sp. and other gastric *Helicobacter* species. Bootstrap
357 values are indicated.

358

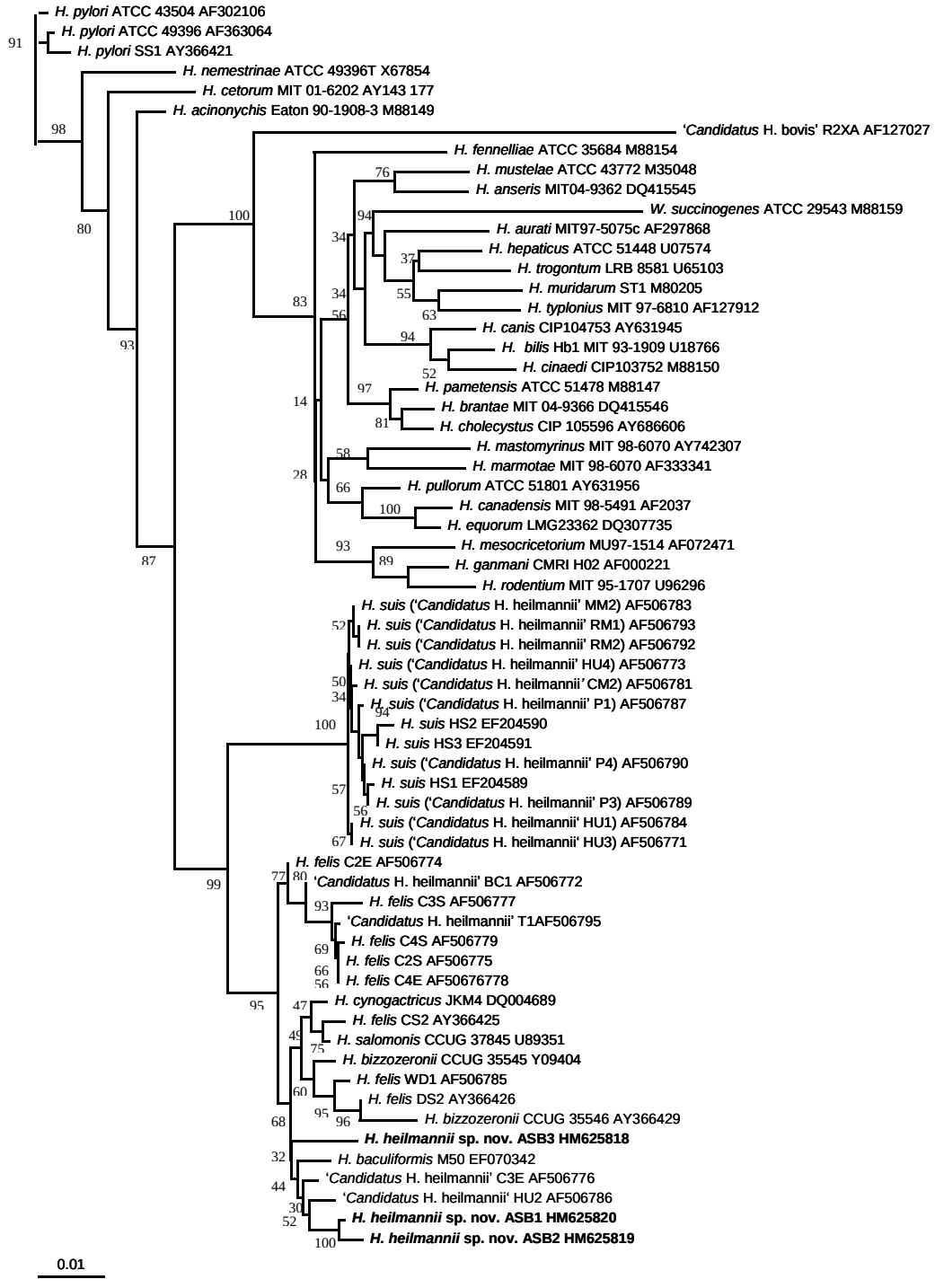
359 **Fig. 4.** TEM images of cells of *H. heilmannii* strain ASB1^T.
360 a, negatively stained cell of strain ASB1^T; b, negatively stained cell of strain ASB1^T showing
361 bipolar flagellae; c, TEM image of strain ASB1^T showing an unusual long cell with up to 9
362 turns; d, negatively stained cell of strain ASB1^T with blunt-ended flagellae; e, TEM image of
363 strain ASB1^T showing a cross section of the flagellae (arrow); f, negatively stained cell of
364 strain ASB1^T showing sheated flagellae. Bars: a, 2µm; b, c and f, 1µm; d, 500nm; e, 200nm.

365

366 **Fig. S1.** Dendrogram derived from the numerical analysis of the whole cell protein profiles of
367 strains ASB1^T, ASB2 and ASB3 and gastric *Helicobacter* reference strains. The asterisk
368 indicates the pattern obtained after growth of the strain on *Brucella* agar supplemented with
369 20% fetal calf serum.

370 **Fig. 1**

371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H. acinonychis Eaton 90-1908-3 M88149
'Candidatus H. bovis' R2XA AF127027
H. fennelliae ATCC 35684 M88154
H. mustelae ATCC 43772 M35048
H. anseris MIT04-9362 DQ415545
W. succinogenes ATCC 29543 M88159
H. aurati MIT97-5075c AF297868
H. hepaticus ATCC 51448 U07574
H. trogontum LRB 8581 U65103
H. muridarum ST1 M80205
H. typlonius MIT 97-6810 AF127912
H. canis CIP104753 AY631945
H. bilis Hb1 MIT 93-1909 U18766
H. cinaedi CIP103752 M88150
H. pametensis ATCC 51478 M88147
H. brantae MIT 04-9366 DQ415546
H. cholecystus CIP 105596 AY686606
H. mastomyrinus MIT 98-6070 AY742307
H. marmotae MIT 98-6070 AF333341
H. pullorum ATCC 51801 AY631956
H. canadensis MIT 98-5491 AF2037
H. equorum LMG23362 DQ307735
H. mesocricetorium MU97-1514 AF072471
H. ganmani CMRI H02 AF000221
H. rodentium MIT 95-1707 U96296
H. suis ('Candidatus H. heilmannii' MM2) AF506783
H. suis ('Candidatus H. heilmannii' RM1) AF506793
H. suis ('Candidatus H. heilmannii' RM2) AF506792
H. suis ('Candidatus H. heilmannii' HU4) AF506773
H. suis ('Candidatus H. heilmannii' CM2) AF506781
H. suis ('Candidatus H. heilmannii' P1) AF506787
H. suis HS2 EF204590
H. suis HS3 EF204591
H. suis ('Candidatus H. heilmannii' P4) AF506790
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H. suis ('Candidatus H. heilmannii' P3) AF506789
H. suis ('Candidatus H. heilmannii' HU1) AF506784
H. suis ('Candidatus H. heilmannii' HU3) AF506771
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'Candidatus H. heilmannii' BC1 AF506772
H. felis C3S AF506777
'Candidatus H. heilmannii' T1AF506795
H. felis C4S AF506779
H. felis C2S AF506775
H. felis C4E AF50676778
H. cynogactricus JKM4 DQ004689
H. felis CS2 AY366425
H. salomonis CCUG 37845 U89351
H. bizzozeronii CCUG 35545 Y09404
H. felis WD1 AF506785
H. felis DS2 AY366426
H. bizzozeronii CCUG 35546 AY366429
H. heilmannii sp. nov. ASB3 HM625818
H. baculiformis M50 EF070342
'Candidatus H. heilmannii' C3E AF506776
'Candidatus H. heilmannii' HU2 AF506786
H. heilmannii sp. nov. ASB1 HM625820
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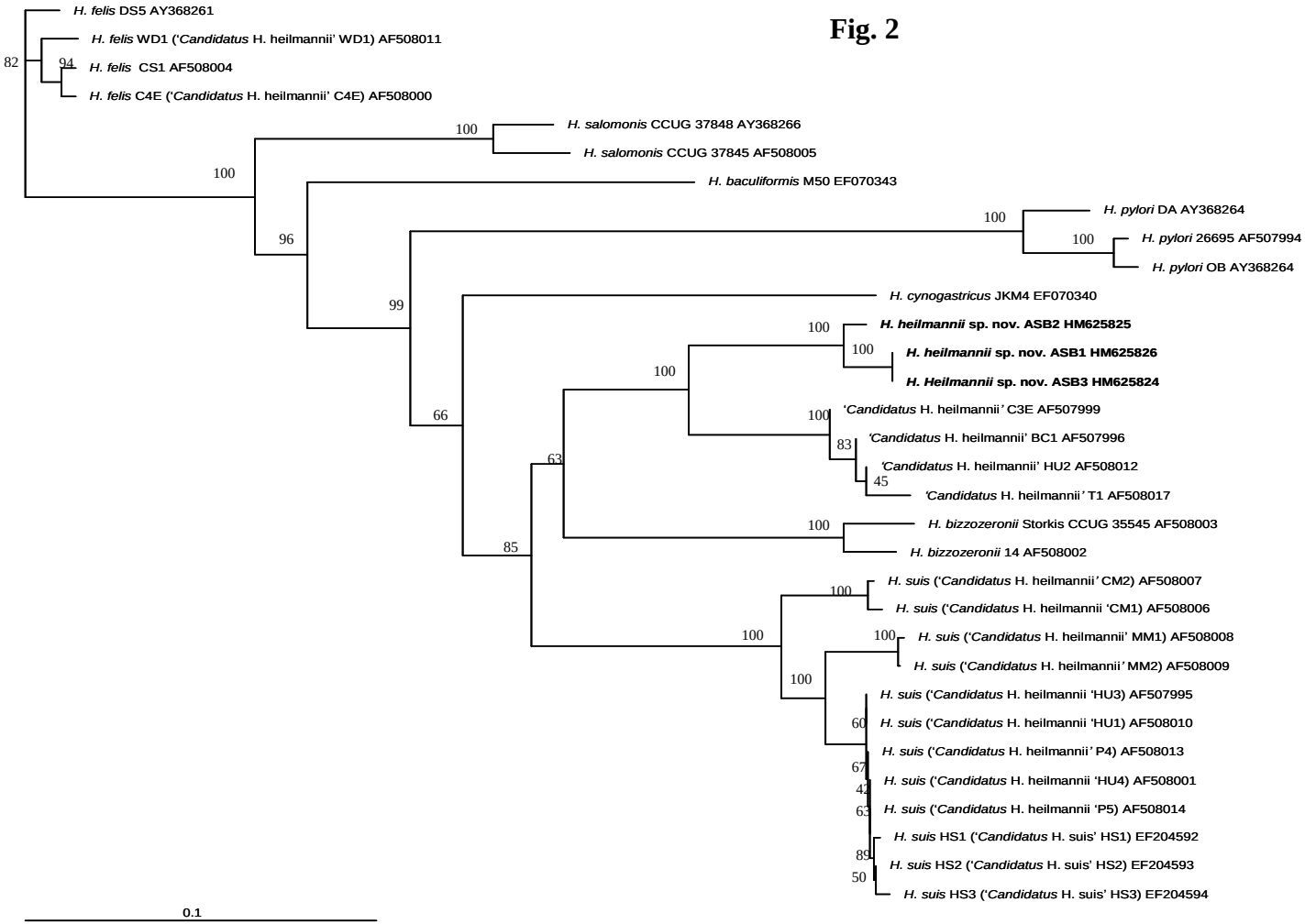
0.01

Fig. 2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H. salomonis CCUG 37845 AF508005
H. baculiformis M50 EF070343
H. pylori DA AY368264
H. pylori 26695 AF507994
H. pylori OB AY368264
H. cynogastricus JKM4 EF070340
***H. heilmannii* sp. nov. ASB2 HM625825**
***H. heilmannii* sp. nov. ASB1 HM625826**
***H. Heilmannii* sp. nov. ASB3 HM625824**
'*Candidatus* H. heilmannii' C3E AF507999
'*Candidatus* H. heilmannii' BC1 AF507996
'*Candidatus* H. heilmannii' HU2 AF508012
'*Candidatus* H. heilmannii' T1 AF508017
H. bizzozeronii Storkis CCUG 35545 AF508003
H. bizzozeronii 14 AF508002
H. suis ('*Candidatus* H. heilmannii' CM2) AF508007
H. suis ('*Candidatus* H. heilmannii 'CM1) AF508006
H. suis ('*Candidatus* H. heilmannii' MM1) AF508008
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H. suis ('*Candidatus* H. heilmannii 'HU3) AF507995
H. suis ('*Candidatus* H. heilmannii 'HU1) AF508010
H. suis ('*Candidatus* H. heilmannii' P4) AF508013
H. suis ('*Candidatus* H. heilmannii 'HU4) AF508001
H. suis ('*Candidatus* H. heilmannii 'P5) AF508014
H. suis HS1 ('*Candidatus* H. suis' HS1) EF204592
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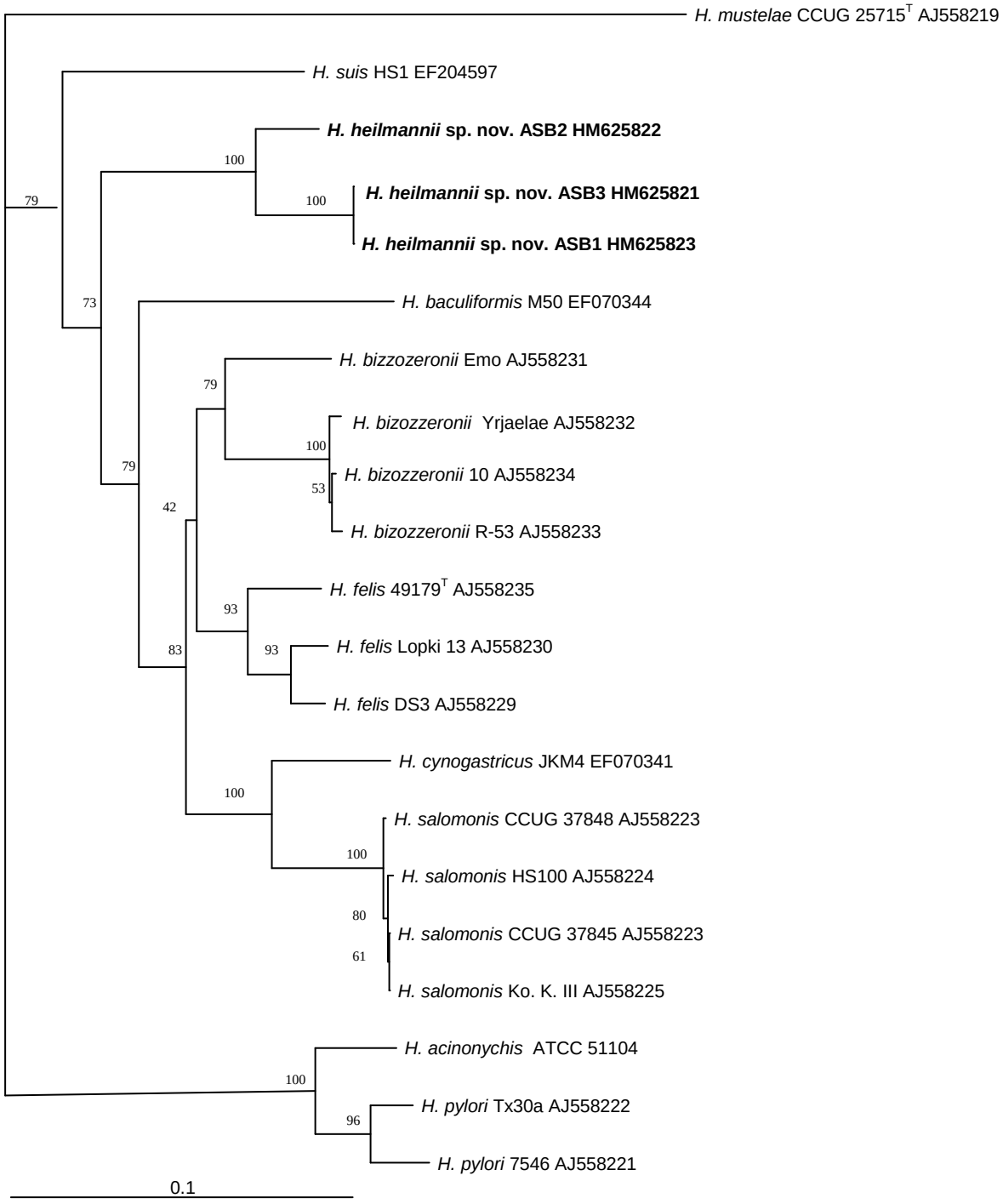
Fig. 3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H. suis HS1 EF204597
***H. heilmannii* sp. nov. ASB2 HM625822**
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***H. heilmannii* sp. nov. ASB1 HM625823**
H. baculiformis M50 EF070344
H. bizzozeronii Emo AJ558231
H. bizozzeronii Yrjaelae AJ558232
H. bizozzeronii 10 AJ558234
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H. felis Lopki 13 AJ558230
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H. cynogastricus JKM4 EF070341
H. salomonis CCUG 37848 AJ558223
H. salomonis HS100 AJ558224
H. salomonis CCUG 37845 AJ558223
H. salomonis Ko. K. III AJ558225
H. acinonychis ATCC 51104
H. pylori Tx30a AJ558222
H. pylori 7546 AJ558221
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