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Mirajkar, N.S., Phillips, N.D., La, T., Hampson, D.J. and Gebhart, C.J. (2016) Characterization and recognition of Brachyspira hampsonii sp. Nov., a novel intestinal spirochete that is pathogenic to pigs. Journal of Clinical Microbiology, 54 (12). pp. 2942-2949.

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JCM Accepted Manuscript Posted Online 14 September 2016
J. Clin. Microbiol. doi:10.1128/JCM.01717-16
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1 **Characterization and recognition of *Brachyspira hampsonii* sp. nov., a novel**
2 **intestinal spirochete that is pathogenic to pigs**

3

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9

10 **RUNNING TITLE: Characterization of *Brachyspira hampsonii* sp. nov.**

11

12 **KEYWORDS:**

13 *Brachyspira*, *hampsonii*, *Brachyspira hampsonii* sp. nov., swine dysentery, species, genomovar,
14 taxonomy, genome, morphology, electron microscopy, DNA-DNA hybridization, Average
15 Nucleotide Identity, Average Amino acid Identity, genotype, phenotype, spirochete, pig

16

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20 **ABSTRACT**

21 Swine dysentery (SD) is a mucohemorhagic colitis of swine classically caused by infection with
22 the intestinal spirochete *Brachyspira hyodysenteriae.* Since around 2007, cases of SD have
23 occurred in North America associated with a different strongly beta-hemolytic spirochete that
24 has been molecularly and phenotypically characterized and provisionally named "*Brachyspira*
25 *hampsonii*". Despite increasing international interest, "*B. hampsonii*" is currently not recognized
26 as a valid species. To support its recognition, we sequenced the genomes of strains NSH-16,
27 NSH-24 and P280/1, representing "*B. hampsonii*" genetic groups I, II and III, respectively, and
28 compared them with genomes of other valid *Brachyspira* species. The draft genome of strain
29 NSH-16 has a DNA G+C content of 27.4% and an approximate size of 3.2 Mb. Genomic indices
30 including digital DNA-DNA hybridization (dDDH), Average Nucleotide Identity (ANI) and
31 Average Amino Acid Identity (AAI) clearly differentiated "*B. hampsonii*" from other recognized
32 *Brachyspira* species. Although discriminated genotypically, the three genetic groups remain
33 phenotypically similar. By electron microscopy, cells of different strains of "*B. hampsonii*"
34 measure 5-10 µm x 0.28-0.34 µm, with one or two flat curves, and have 10 to 14 periplasmic
35 flagella inserted at each cell end. Using a comprehensive evaluation of genotypic (gene
36 comparisons and multi-locus sequence typing and analysis), genomic (dDDH, ANI and AAI) and
37 phenotypic (hemolysis, biochemical profiles, protein spectra, antibiogram and pathogenicity)
38 properties, we classify *Brachyspira hampsonii* sp. nov. as a unique species with genetically
39 diverse yet phenotypically similar 'genomovars' (I, II and III). We designate the type strain as
40 NSH-16^{T} (=ATCC® BAA-2463™ = NCTC 13792).

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42 **INTRODUCTION**

43 The genus *Brachyspira* includes Gram-negative, aerotolerant, anaerobic spirochetes that colonize
44 the intestine of and/or cause disease in a wide range of host species (1). Over several decades,
45 multiple taxonomic changes were applied to members of this genus (originally *Treponema*, then
46 transferred to *Serpula*, then to *Serpulina* and finally to *Brachyspira*) (2–5). Currently, the genus
47 *Brachyspira* consists of eight valid species including *B. hyodysenteriae*, *B. pilosicoli*, *B.*
48 *intermedia*, *B. innocens*, *B. murdochii*, *B. aalborgi*, *B. alvinipulli*, and most recently, *B.*
49 *suanatina* (1, 6). This genus also consists of several provisional species (1), of which the most
50 clinically significant is the recently discovered "*B. hampsonii*" (7). Within the *Brachyspira*
51 genus, all currently identified strongly beta-hemolytic species (*B. hyodysenteriae*, *B. suanatina*
52 and the novel "*B. hampsonii*") are known to cause severe mucohemorrhagic diarrhea, while
53 weakly beta-hemolytic *Brachyspira* species are either commensals (*B. innocens*) or are capable
54 of causing diarrhea and/or colitis (*B. pilosicoli*, *B. murdochii*, *B. intermedia*, *B. aalborgii* and *B.*
55 *alvinipulli*) (1). *B. hyodysenteriae*, the most virulent and clinically significant *Brachyspira*
56 species, has historically also been the most researched or investigated species. It causes swine
57 dysentery (SD), a disease characterized by mucohemorrhagic diarrhea that is most commonly
58 observed in grower-finisher pigs (1). In addition to the adverse impact on the health and welfare
59 of pigs, its negative effect on productivity (such as decreased weight gain and poor feed
60 conversion) leads to significant economic losses to livestock-raising communities and countries
61 (1). The recently validated *B. suanatina* also causes SD in pigs; however, its isolation has been
62 limited to a few northern European countries (8). The isolation of different bacterial species from
63 clinically and pathologically indistinguishable dysentery cases of pigs highlights the evolving
64 and expanding etiology of SD. Thus, the definition of SD should include all strongly beta-

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65 hemolytic *Brachyspira* species that cause mucohemorrhagic colitis and dysentery in pigs (9).
66 The genetically diverse *B. pilosicoli* is the primary etiological agent of colonic spirochetosis, a
67 disease characterized by diarrhea and/or colitis in a wide range of host species including pigs
68 (Porcine Intestinal Spirochetosis - PIS) (10), chickens (Avian Intestinal Spirochetosis - AIS) (11)
69 and human beings (Human Intestinal Spirochetosis - HIS) (12). AIS also can be caused by other
70 *Brachyspira* species including *B. intermedia* (13) and *B. alvinipulli* (14), while HIS is also
71 caused by *B. aalborgi* (15). Although long considered to be a commensal, the association of *B.*
72 *murdochii* with mild diarrhea and/or colitis in pigs has been reported (16, 17). These
73 *Brachyspira*-associated disease conditions negatively impact the health and welfare of the
74 affected host species and reduce the productivity of livestock (1).

75 Clinical SD was rarely reported in North America after the early 1990s, despite continuing to
76 have negative impacts on the health and productivity of pigs in other countries across the world.
77 Outbreaks of bloody diarrhea in commercial swine herds in 2007 signaled the re-emergence of
78 this disease in North America (18). Interestingly, the detection of re-emergent *B. hyodysenteriae*
79 in the US was accompanied by the unexpected discovery of a novel species "*B. hampsonii*" from
80 cases of classic mucohemorrhagic diarrhea that were clinically indistinguishable from those
81 caused by *B. hyodysenteriae*. Preliminary characterization lead to the identification and
82 provisional designation of *"B. hampsonii"* and its two diverse genetic groups (previously called
83 clades) - group I and group II (7). Since the initial identification of "*B. hampsonii*" in North
84 American pigs, it has also been detected in pigs in Belgium and Germany (19, 20) and in
85 migratory water birds in Europe and North America (21, 22).

86 Several methods have been used to characterize the phenotype of "*B. hampsonii*" including
87 growth characterization, identification and qualification of hemolysis on blood agar, biochemical

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88 tests (hippurate hydrolysis, production of indole, α-galactosidase, α-glucosidase and β-
89 glucosidase activities), protein spectra profiling, antibiogram testing and characterization of its
90 pathogenic nature. Growth on solid media (tryptic soy agar containing 5% defibrinated sheep
91 blood) is observed as tiny transparent colonies with underlying strong beta-hemolysis (23) that is
92 most distinct in areas of cuts made in the agar (known as the "ring phenomenon"), while growth
93 in liquid media (brain-heart infusion broth supplemented with 10% fetal bovine serum) is
94 observed as light turbidity in the broth (24). Cultural properties alone are insufficient to
95 differentiate "*B. hampsonii*" from other strongly beta-hemolytic *Brachyspira* species (*B.*
96 *hyodysenteriae* and *B. suanatina*), thus emphasizing the need for other phenotypic or genotypic
97 tests. "*B. hampsonii*" isolates were found to be negative for indole production, as well as
98 hippurate, α-galactosidase and α-glucosidase activity, with the indole spot test being the most
99 useful test for differentiating "*B. hampsonii*" from other strongly beta-hemolytic *Brachyspira*
100 species (*B. hyodysenteriae* and *B. suanatina* are usually indole positive) (7, 25). Although two
101 biochemical profiles for "*B. hampsonii*" have been described, neither was absolutely effective in
102 differentiating genetic groups I and II (7). Main spectra profiles (MSPs) generated with the
103 matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS)
104 technology were consistently able to identify "*B. hampsonii*" and differentiate "*B. hampsonii*"
105 from other *Brachyspira* species (26). Although this method was able to often differentiate
106 between the genetic groups of "*B. hampsonii*", this differential identification was not consistently
107 reliable (26). A study characterizing antibiograms of North American "*B. hampsonii*" isolates
108 demonstrated high susceptibility to several commonly used antimicrobials including tiamulin,
109 valnemulin, lincomycin, tylosin, doxycycline and carbadox (27). Although "*B. hampsonii*" often
110 demonstrated a more susceptible antibiogram compared to other *Brachyspira* species, no clear

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111 differences in antibiogram profiles were observed between its genetic groups (27). Finally,
112 several trials have reproduced mucohemorrhagic diarrhea in pigs by oral inoculation of "*B.*
113 *hampsonii*" genetic groups I and II under experimental conditions and have thus confirmed the
114 pathogenic nature of both groups (28–30). The resulting disease was indistinguishable from SD
115 caused by *B. hyodysenteriae* on the basis of clinical signs and gross pathology. Examination of
116 tissues obtained from the experimentally infected pigs had microscopic lesions consistent with
117 those seen in the mucohemorrhagic colitis induced by *B. hyodysenteriae* (28–30). Currently, no
118 differences in clinical signs or gross and microscopic pathology have been reported in the SD
119 caused by either genetic groups I or II of "*B. hampsonii*".

120 Several methods have been used to characterize the genotype of "*B. hampsonii*" including gene
121 comparisons and identification of genotypes (7, 31). For the purpose of species delineation in the
122 *Brachyspira* genus, the NADH oxidase (*nox*) gene has historically been considered to be more
123 useful than the 16S ribosomal RNA gene (32). Both of these genes have been used to identify
124 "*B. hampsonii*" and differentiate it from other *Brachyspira* species, as well as to differentiate
125 between the diverse genetic groups of "*B. hampsonii*" (7). The *nox* gene is also often used as a
126 target for diagnostic tests such as qPCR and Sanger sequencing (7, 29) to specifically detect "*B.*
127 *hampsonii*". Genotyping of "*B. hampsonii*" from diverse epidemiological origins using the multi-
128 locus sequence typing (MLST) approach (31) identified a total of 20 genotypes that clustered
129 into four genetic groups (I, II, III and IV). It included the commonly reported genetic groups I
130 and II that are frequently isolated from affected North American pigs (7), and occasionally
131 isolated from pigs in Europe (19, 20) and from migratory birds of both North American and
132 European origin (21, 22). It also included the less frequently reported genetic group III which has
133 been isolated occasionally from pigs and migratory water birds of European origin (20, 21, 33),

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134 as well as the rare genetic group IV which has only been detected in migratory water birds in
135 Europe (21). Overall "*B. hampsonii*" was observed to demonstrate high diversity and a
136 heterogeneous population structure (31). In addition, a *Brachyspira* genus-wide multi-locus
137 sequence analysis (MLSA) approach was used to confirm that "*B. hampsonii*" could be
138 differentiated from other *Brachyspira* species. This study reported clustering of "*B. hampsonii*"
139 genetic groups in spite of its diverse nature (31).

140 Despite the significance of this novel pathogenic species and the information that is currently
141 available, "*B. hampsonii*" is still classified as a proposed species. Therefore the objective of this
142 study is to support its position as a valid species by providing additional information on its whole
143 genome sequences, genomic relatedness to other *Brachyspira* species and ultrastructural
144 morphology.

145

146 **MATERIALS & METHODS**

147 **"*B. hampsonii*" isolates:** "*Brachyspira hampsonii*" strains NSH-16 (ATCC® BAA-2463™),
148 NSH-24 (ATCC® BAA-2464™) and P280/1 were selected for study as they represent the type
149 strains for genetic groups I, II and III, respectively. Most importantly, strain NSH-16 is also the
150 designated type strain for "*B. hampsonii*" (ATCC® BAA-2463™ = NCTC 13792). Plates of
151 tryptic soy agar (TSA) (BD, Franklin Lakes, NJ, USA) containing 5% defibrinated sheep blood
152 (I-Tek Medical Technologies, MN, USA) were inoculated with pure cultures and incubated
153 under anaerobic conditions at 37°C for four days. Growth was observed as zones of strong beta-
154 hemolysis with observation of the characteristic ring phenomenon. The purity of the isolates was
155 confirmed by phase-contrast microscopy of wet mounts.

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156 **Phase Contrast and Electron Microscopy:** Cells of strains NSH-16, NSH-24 and P280/1 were
157 grown to mid log phase on TSA plates and prepared for phase contrast and electron microscopy
158 as described previously (10). Actively dividing cells were gently harvested from the plates with 1
159 mL 0.01 M sodium phosphate buffer at pH 7.0 and centrifuged at 2,000 × g for 3 min. The pellet
160 was resuspended with 1 ml phosphate buffer and centrifuged at 2,000 × g to wash the cells.
161 Washing was performed three times before resuspending the cells with 0.5 mL phosphate buffer.
162 Washed cells were adhered to coverslips using 0.1% polyethyleneimine and examined with a
163 Nikon ECLIPSE 90i microscope under a 100X phase contrast objective with a Ph3 condenser
164 ring. A 0.02 mL sample of the washed cells was negatively stained with an equal volume of 2%
165 phosphotungstic acid (pH 7) before being mounted on a carbon-reinforced 200-mesh copper grid
166 coated with 2% Parlodion. The grids were examined with a Phillips model 410 transmission
167 electron microscope. Cell dimensions and the ultrastructural characteristics of the spirochete
168 were determined from electron micrographs of at least ten individual cells.

169 **Genome sequencing, assembly and annotation:** Genomic DNA from each isolate was
170 extracted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) as per the
171 manufacturer's instructions. For strains NSH-16 and NSH-24, the quality control, library
172 preparation and whole genome sequencing of the extracted genomic DNA was carried out at the
173 University of Minnesota Genomics Center, Minneapolis. Briefly, the samples were evaluated for
174 quality control and DNA concentrations using the Quant-iT™ PicoGreen® dsDNA Assay Kit
175 (Thermo Fisher Scientific, Waltham, MA, USA) and the DNA library was prepared using the
176 Nextera XT DNA Library Preparation Kit (Illumina, CA, USA) as per the manufacturer's
177 instructions. Sequencing was carried out using MiSeq Reagent Kit V3 (Illumina, CA, USA) with
178 a paired end 2x300 bp construct on the MiSeq system (Illumina, CA, USA). This yielded

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179 4,566,767 and 3,603,642 passing filter reads for strains NSH-16 and NSH-24 which
180 corresponded to an average genome coverage of approximately 860X and 700X, respectively.
181 Using the default parameters of the De Novo Assembly tool of CLC Genomics Grid Workbench
182 8.0.2, the reads were quality checked, trimmed based on quality, and assembled *de novo* to
183 generate contigs. Filters were applied to select and extract a subset of contigs with consensus
184 length ≥ 1 kb and coverage ≥ 50X in order to generate a draft genome. Whole genome
185 sequencing of strain P280/1 was performed in Australia by Geneworks Pty Ltd (Thebarton, SA,
186 Australia) under the Illumina Certified Service Provider (CSPro) Program. Sequencing was
187 carried out using the TruSeq DNA PCR-Free Library Preparation Kit (Illumina, CA, USA) with
188 a paired end 2x75 bp construct on the Genome Analyzer IIx (Illumina, CA, USA), which yielded
189 14,097,542 reads corresponding to an average genome coverage of approximately 661X. *De*
190 *novo* assembly of reads was performed with SeqMan NGen 3.0 Assembly Software (DNASTAR,
191 Madison, WI, USA) using default parameters to generate a draft genome. All three strains were
192 annotated using the Rapid Annotation using Subsystems Technology (RAST version 2.0) (34)
193 with parameters allowing for frameshift error corrections. The genomes were also annotated
194 using the NCBI Prokaryotic Genome Annotation Pipeline (35).

195 **Genome comparisons for species delineation:** Publicly available genomes of various
196 *Brachyspira* species were obtained from the NCBI genome database
197 (https://www.ncbi.nlm.nih.gov/genome/). These included *B. hyodysenteriae* (strain B-78^T and
198 strain WA1), *B. suanatina* strain AN4859/03^T, *B. pilosicoli* strain P43/6/78^T, *B. intermedia* strain
199 PWS/A^T, *B. murdochii* strain 56-150^T, *B. innocens* strain B256^T, *B. alvinipulli* strain 911207/C1^T
200 and "*B. hampsonii*" (strain 30599 and strain 30446). The publicly available genome of *B.*
201 *aalborgii* strain 513A^T was obtained from MetaHIT Consortium website

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202 (http://www.sanger.ac.uk/resources/downloads/bacteria/metahit/). The Genome to Genome
203 Distance (GGD) values of "*B. hampsonii*" strains NSH-16, NSH-24 and P280/1 and other
204 *Brachyspira* species and strains were calculated using the Genome-to-Genome Distance
205 Calculator (GGDC 2.1) web service (http://ggdc.dsmz.de/distcalc2.php) (36). Similarly, the
206 average nucleotide identity (ANI) values and the average amino acid identity (AAI) values of "*B.*
207 *hampsonii*" strains NSH-16, NSH-24 and P280/1 and other *Brachyspira* species and strains were
208 calculated using the EzGenome ANI web service (http://www.ezbiocloud.net/ezgenome/ani) (37)
209 based on the algorithm of Goris et al. (38), and using the web-based AAI tool (http://enve-
210 omics.ce.gatech.edu/aai/index) (39) based on two-way AAI calculations, respectively. SpecI, a
211 web-based species identification tool (http://vm-lux.embl.de/~mende/specI/) (40) was used to
212 extract 40 universal single copy marker genes of "*B. hampsonii*" strains NSH-16, NSH-24 and
213 P280/1 and evaluate the average genetic distance of these strains from publicly available
214 complete genomes of valid bacterial species.

215

216 **RESULTS**

217 By phase contrast and electron microscopy the shape of the spirochete cells was consistent with
218 that of other *Brachyspira* species. Cells had slightly tapered ends and one or two flat serpentine
219 curves (Figure 1). The cells of P280/1 were longer than those of NSH-16 and NSH-24, but were
220 otherwise similar, with 10 to 14 periplasmic flagella inserted sub-terminally at each end of the
221 cell, with a total of 20 to 28 flagella per cell (Figure 2). Cells of P280/1 were 10.49 ± 0.41 µm
222 long, whereas those of NSH-16 and NSH-24 were 5.43 ± 0.34 and 5.06 ± 0.37 µm long
223 respectively (Table 1). Mean cell widths for the strains varied from 0.28 to 0.34 µm.

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224 The final assembly of "*B. hampsonii*" strain NSH-16 genome resulted in 77 contigs comprising
225 approximately 3.16 Mb with a G+C content of 27.4%. Eleven large contigs >100 kb in size and
226 another 43 contigs 10-100 kb in size comprised 97.4% of the assembled *B. hampsonii*" strain
227 NSH-16 genome. The final assembly of "*B. hampsonii*" strain NSH-24 resulted in 178 contigs
228 comprising approximately 2.97 Mb with a G+C content of 27.5%. One large contig >100 kb in
229 size and another 93 contigs 10-100 kb in size comprised 88% of the assembled *B. hampsonii*"
230 strain NSH-24 genome. Assembly of the "*B. hampsonii*" strain P280/1 genome resulted in 16
231 contigs of 3,186,631 bp, with a G+C content of 27.5%. The general genomic features of *B.*
232 *hampsonii*" strains NSH-16, NSH-24 and P280/1 are described in Table 2.

233 The GGD, ANI and AAI values comparing "*B. hampsonii*" strains NSH-16, NSH-24 and P280/1
234 with other *Brachyspira* species and strains are described in Tables 3, 4 and 5, respectively.
235 Comparison of "*B. hampsonii*" GGD values with other *Brachyspira* species, between "*B.*
236 *hampsonii*" genetic groups and within "*B. hampsonii*" genetic groups were approximately ~20-
237 35%, ~50-57% and ~99%, respectively. A similar trend was observed when using the ANI
238 method, where inter-species, inter-genetic group and intra-genetic group comparison yielded
239 nucleotide identities of approximately ~75-88%, ~93-94.5% and ~100%, respectively. The AAI
240 method yielded mostly similar results for inter-species, inter-genetic group and intra-genetic
241 group comparisons with amino acid identities of approximately ~72-90%, ~94-95% and ~100%,
242 respectively. SpecI was unable to categorize "*B. hampsonii*" as any previously recognized valid
243 bacterial species. Interestingly, it identified "*B. hampsonii*" genetic group I as a closest match to
244 *B. hyodysenteriae*, and genetic groups II and III as closest matches to *B. intermedia*.

245

246 **DISCUSSION**

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247 Since the initiation of bacterial taxonomy in the late 19th century, the accepted taxonomic
248 practices for delineation of novel species have evolved with the advent of new technologies and
249 scientific methods. Initially bacteria were classified on phenotypic characteristics such as growth
250 requirements, morphology, pathogenicity, physiology, and biochemical activity. Gradually
251 chemotaxonomy, numerical taxonomy, conventional DDH, DNA G+C content and eventually
252 16S ribosomal RNA gene sequencing provided further methods of species differentiation. A
253 detailed review of the history of bacterial taxonomy has been provided by Schleifer KH (41).
254 Most recently, whole genome sequencing has facilitated several additional approaches to species
255 delineation including comparison of genome indexes, gene content and multiple gene aligned
256 sequence datasets (42). The utility of DNA G+C content comparison is limited as members of
257 several bacterial genera show high conservation of G+C content, and thus this method serves
258 mostly as an exclusionary determinant (41). Of the mentioned genotypic methods, conventional
259 DDH and 16S rRNA gene sequencing have been widely used for differentiating bacterial species
260 over the last several years (42). Although 16S rRNA gene sequencing is an effective way to
261 differentiate bacterial species because of its genetically and functionally highly stable nature, this
262 method is not useful for some bacterial species that have multiple rRNA operons in a single
263 genome or show a high degree of conservation within a genus (41). Further, conventional DDH
264 is known to be laborious, error-prone with low reproducibility, expensive and not equally
265 applicable to all bacterial genera (41). Thus methods evaluating whole genome sequence
266 similarity such as digital DDH were proposed as they overcome many of the drawbacks while
267 maintaining a good correlation with conventional DDH and 16S rRNA sequencing for species
268 delineation (38, 43). Given the plethora of methods available, current prokaryotic taxonomy is

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269 often based on polyphasic combinations of phenotypic, genotypic, genomic and/or
270 chemotaxonomic characteristics (41).

271 In the case of "*B. hampsonii*" no completely distinctive phenotypical differences from all strains
272 of other valid *Brachyspira* species have been found to date, and this study confirms that even the
273 ultrastructure of "*B. hampsonii*" cells is similar to that of some other *Brachyspira* species, such
274 as *B. hyodysenteriae*. The genomes of "*B. hampsonii*" strains NSH-16, NSH-24 and P280/1
275 show similar G+C content, which falls within the general range of G+C content currently
276 identified for members of the *Brachyspira* genus (~27% to 28%). This is not surprising, as
277 diverse *Brachyspira* species show limited variation in their average chromosomal G+C content
278 (6, 44–47). The approximated genome size also falls within the range of most members of the
279 *Brachyspira* genus (range: ~2.7 Mb to ~3.4 Mb) (6, 44–47). Applying the recommended <70%
280 threshold value for DDH (48) to GGD results, and the <95-96% threshold (49, 50) to ANI and
281 AAI results, these "*B. hampsonii*" strains did not fall under the classification of any previously
282 recognized *Brachyspira* species. Further, the 96.5% threshold for similarity to universal marker
283 genes (40) was also unable to assign these strains to any known bacterial or archaeal species.
284 These genomic indices add to the already existing information supporting the position of "*B.*
285 *hampsonii*" as a novel species. Surprisingly, based upon several universal marker genes, the
286 closest matches identified for the various genetic groups differed (i.e. *B. hyodysenteriae* for
287 genetic group I and *B. intermedia* for genetic groups II and II). A similar observation was made
288 by the use of whole-genome sequence data, wherein "*B. hampsonii*" showed the closest identity
289 to *B. hyodysenteriae*, *B. intermedia* and *B. suanatina*, followed by *B. murdochii* and *B. innocens*.
290 This was in contrast to previous studies (7, 21, 31) which identified "*B. hampsonii*" to be most
291 genetically related to *B. murdochii* and/or *B. innocens*. Since those studies (7, 21, 31) evaluated

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292 only a few conserved genes, it is likely that the genetic relatedness of the overall genome was
293 under-represented. The use of whole-genome data in this study provides the opportunity to make
294 more detailed and extensive comparisons between "*B. hampsonii*" and other species. Future
295 studies comparing the core genomes of various *Brachyspira* species will help to identify which
296 of these species "*B. hampsonii*" shares common ancestors with.

297 The genus *Brachyspira* is unique and complicated as it consists of a variety of species that can
298 infect a wide range of host species with different abilities to cause disease, yet each shows
299 varying degrees of ability to be differentiated by phenotypic and genotypic characteristics. For
300 instance, the low variation in 16S rRNA gene sequence and DNA G+C content (<1%) would be
301 insufficient to differentiate the various species within the *Brachyspira* genus. On the other hand,
302 genetically and phenotypically diverse species (*B. hyodysenteriae*, *B. suanatina* and "*B.*
303 *hampsonii*") all infect a single host species (pig), occupy the same ecological niche (the colon)
304 and cause a clinically and pathologically indistinguishable disease (SD). Thus a comprehensive
305 and conservative approach that evaluates information on a variety of genotypic and phenotypic
306 properties as well as ecological characteristics should be applied in delineating species within the
307 *Brachyspira* genus. While both genotypic and phenotypic data clearly support "*B. hampsonii*" as
308 a novel species, they provide ambiguous interpretations for whether the various genetic groups
309 represent one or multiple novel species. Specifically, although the genomic indices (GGD, ANI
310 and AAI values) comparing "*B. hampsonii*" genetic groups I, II and III to each other are
311 significantly lower than the threshold of species differentiation, they are also significantly higher
312 than the values obtained when comparing either of the genetic groups with other *Brachyspira*
313 species. This depicts a situation wherein based on genomic information, one could identify the
314 genetic groups of "*B. hampsonii*" as three closely related species. Tindall et al. (42) recommends

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315 that the <70% threshold for DDH (and by correlation other genome sequence identity methods)
316 should not be used as a strict boundary for species delineation. A species can include strains with
317 DDH values <50% if these strains are not clearly distinguishable based on other properties such
318 as phenotypic characteristics (42). Ursing et al. (51) recommends that such genomic groups be
319 classified as 'genomovars' of a single species, with the possibility for reclassification as different
320 species once clear and stable discriminative phenotypic properties are identified. Although the
321 genotypic properties (i.e. gene sequence comparisons (7), MLST (31), MLSA (31), GGD, ANI
322 and AAI) reliably discriminate several genetic groups of "*B. hampsonii*", currently, analysis of
323 the available phenotypic properties (i.e. beta-hemolysis on blood agar (7), biochemical profiles
324 (7), MALDI protein spectra (26), antibiograms (27) and pathogenicity (28–30)) is unable to
325 clearly and consistently differentiate them. Thus, based on a comprehensive genotypic,
326 phenotypic and genomic evaluation we propose that *Brachyspira hampsonii* sp. nov. should be
327 considered a single novel species with multiple genomovars. To that effect, the various "*B.*
328 *hampsonii*" genetic groups (31) (previously called clades (7)) should henceforth be referred to as
329 'genomovars', such that genetic groups I, II and III be replaced by the terms genomovars I, II
330 and III, respectively.

331

332 **Description of *Brachyspira hampsonii* sp. nov.**

333 *Brachyspira hampsonii* (hamp.so'ni.i N.L. masc. gen. n. hampsonii of Hampson), in recognition
334 of Dr. David J. Hampson for his extensive work on the *Brachyspira* genus, as first proposed by
335 Chander et al. (7).

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336 *Brachyspira hampsonii* sp. nov. is a Gram-negative, oxygen-tolerant anaerobe and strongly beta-
337 hemolytic spirochete. *B. hampsonii* cells measure 5-10 µm x 0.25-0.38 µm, have slightly tapered
338 ends, and have one to two flat serpentine coils. Each spirochete cell has 10 to 14 periplasmic
339 flagella inserted at each end of the cell. Growth occurs after inoculated agar (stationary) or broth
340 (rotating at ~80 rpm) has been incubated at 37°C for four days under anaerobic (80% N_2 - 10%
341 CO_2 - 10% H_2) conditions. Growth on tryptic soy agar containing 5% defibrinated sheep blood is
342 observed as tiny transparent colonies with underlying strong beta-hemolysis that is most distinct
343 in areas of cuts made in the agar (known as the 'ring phenomenon'). Growth in brain-heart
344 infusion broth containing 10% fetal bovine serum is observed as light turbidity. Strains are
345 indole negative, hippurate negative, α-galactosidase negative and α-glucosidase negative, and
346 either positive or negative for β-glucosidase. Strains of this species colonize pigs in which they
347 induce swine dysentery characterized by mucohemorrhagic diarrhea. They also are recorded as
348 naturally colonizing species of waterfowl including feral ducks and geese. They are highly
349 susceptible to the antimicrobials tiamulin, valnemulin and carbadox. Strains of this species can
350 be genetically differentiated from other *Brachyspira* species by the use of *nox* gene sequencing,
351 MLST and whole genome sequencing, as well as species-specific PCRs based on the *nox* gene.
352 The draft genome of *B. hampsonii* sp. nov. strain NSH-16 has a DNA G+C content of 27.4% and
353 an approximate genome size of 3.2 Mb. Multiple genotypic (MLST, 16S rRNA and *nox* gene
354 sequence comparisons), genomic (GGD, ANI and AAI) and phenotypic measures (hemolysis,
355 biochemical profiles, MALDI and antibiograms) support the taxonomic classification of
356 *Brachyspira hampsonii* sp. nov. They also support the detection of several genetically diverse yet
357 phenotypically similar groups that have now been designated as genomovars (I, II, and III). The
358 type strain for *Brachyspira hampsonii* sp. nov. is NSH-16^T. The type strains for *B. hampsonii* sp.

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359 nov. genomovar I, *B. hampsonii* sp. nov. genomovar II and *B. hampsonii* sp. nov. genomovar III
360 are designated as NSH-16, NSH-24 and P280/1, respectively. *B. hampsonii* sp. nov. strain NSH-
361 16^T (= ATCC® BAA-2463™ = NCTC 13792) and *B. hampsonii* sp. nov. strain NSH-24 (=
362 ATCC® BAA-2464™ = NCTC 13793) have been deposited with two recognized culture
363 collections in two different countries (ATCC, USA and NCTC, UK).

364 **Accession numbers:**

365 The Whole Genome Shotgun projects for *B. hampsonii* strains NSH-16^T, *B. hampsonii* NSH-24
366 and *B. hampsonii* P280/1 have been deposited at DDBJ/ENA/GenBank under the accessions
367 LZOF00000000, LZOG00000000 and MDCO00000000, respectively. The versions described in
368 this paper are LZOF01000000, LZOG01000000 and MDCO01000000, respectively.

369

370 **ACKNOWLEDGEMENTS**

371 We thank the Minnesota Supercomputing Institute for providing computing resources and Dr.
372 Ying Zhang for her guidance with genome assembly. We thank Dr. Anibal Armien (College of
373 Veterinary Medicine, University of Minnesota) and Dr. Robert Cook of the Faculty of Medicine,
374 Dentistry and Health Sciences at the University of Western Australia for their assistance with the
375 electron microscopy.

376

377 **REFERENCES**

378 1. **Hampson DJ**. 2012. Brachyspiral colitis., p. 680–696. *In* Zimmerman, J, Karriker, L,
379 Ramirez, A, Schwartz, K, Stevenson, G (eds.), Diseases of Swine, 10th ed. Wiley-

Accepted Manuscript Posted Online

380 Blackwell, Chichster, United Kingdom.

381 2. **Harris DL**, **Glock RD**, **Christensen CR**, **Kinyon JM**. 1972. Inoculation of pigs with
382 *Treponema hyodysenteriae* (new species) and reproduction of the disease. Vet Med Small
383 Anim Clin **67**:61–64.

384 3. **Stanton TB**, **Jensen NS**, **Casey TA**, **Tordoff LA**, **Dewhirst FE**, **Paster BJ**. 1991.
385 Reclassification of *Treponema hyodysenteriae* and *Treponema innocens* in a new genus,
386 *Serpula* gen. nov., as *Serpula hyodysenteriae* comb. nov. and *Serpula innocens* comb.
387 nov. Int J Syst Bacteriol **41**:50–58.

388 4. **Stanton TB**. 1992. Proposal to change the genus designation *Serpula* to *Serpulina* gen.
389 nov. containing the species *Serpulina hyodysenteriae* comb. nov. and *Serpulina innocens*
390 comb. nov. Int J Syst Bacteriol **42**:189–90.

391 5. **Ochiai S**, **Adachi Y**, **Mori K**. 1997. Unification of the genera *Serpulina* and *Brachyspira*,
392 and proposals of *Brachyspira hyodysenteriae* comb. nov., *Brachyspira innocens* comb.
393 nov. and *Brachyspira pilosicoli* comb. nov. Microbiol Immunol **41**:445–52.

394 6. **Mushtaq M**, **Zubair S**, **Råsbäck T**, **Bongcam-Rudloff E**, **Jansson DS**. 2015.
395 *Brachyspira suanatina* sp. nov., an enteropathogenic intestinal spirochaete isolated from
396 pigs and mallards: genomic and phenotypic characteristics. BMC Microbiol **15**:208.

397 7. **Chander Y**, **Primus A**, **Oliveira S**, **Gebhart CJ**. 2012. Phenotypic and molecular
398 characterization of a novel strongly hemolytic *Brachyspira* species, provisionally
399 designated “*Brachyspira hampsonii*”. J Vet Diagn Invest **24**:903–10.

400 8. **Rasback T**, **Jansson DS**, **Johansson KE**, **Fellstrom C**. 2007. A novel enteropathogenic,

Journal of Clinical Microbiology
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Journal of Clinical Microbiology
Downloaded from http://jcm.asm.org/ on December 11, 2016 by SUB NO.63745291

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Journal of Clinical Microbiology

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401 strongly haemolytic spirochaete isolated from pig and mallard, provisionally designated
402 "*Brachyspira suanatina*" sp. nov. Environ Microbiol **9**:983–991.

403 9. **Burrough ER**. 2016. Swine Dysentery: etiopathogenesis and diagnosis of a reemerging
404 disease. Vet Pathol.

405 10. **Trott DJ**, **Stanton TB**, **Jensen NS**, **Duhamel GE**, **Johnson JL**, **Hampson DJ**. 1996.
406 *Serpulina pilosicoli* sp. nov., the agent of porcine intestinal spirochetosis. Int J Syst
407 Bacteriol **46**:206–215.

408 11. **McLaren AJ**, **Trott DJ**, **Swayne DE**, **Oxberry SL**, **Hampson DJ**. 1997. Genetic and
409 phenotypic characterization of intestinal spirochetes colonizing chickens and allocation of
410 known pathogenic isolates to three distinct genetic groups. J Clin Microbiol **35**:412–417.

411 12. **Trivett-Moore NL**, **Gilbert GL**, **Law CLH**, **Trott DJ**, **Hampson DJ**. 1998. Isolation of
412 *Serpulina pilosicoli* from rectal biopsy specimens showing evidence of intestinal
413 spirochetosis. J Clin Microbiol **36**:261–265.

414 13. **Hampson DJ**, **McLaren AJ**. 1999. Experimental infection of laying hens with *Serpulina*
415 *intermedia* causes reduced egg production and increased faecal water content. Avian
416 Pathol **28**:113–117.

417 14. **Stanton TB**, **Postic D**, **Jensen NS**. 1998. *Serpulina alvinipulli* sp. nov., a new *Serpulina*
418 species that is enteropathogenic for chickens. Int J Syst Bacteriol **48 Pt 3**:669–676.

419 15. **Mikosza ASJ**, **La T**, **Brooke CJ**, **Lindboe CF**, **Ward PB**, **Heine RG**, **Guccion JG**, **De**
420 **Boer WB**, **Hampson DJ**. 1999. PCR amplification from fixed tissue indicates frequent
421 involvement of *Brachyspira aalborgi* in human intestinal spirochetosis. J Clin Microbiol

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422 **37**:2093–2098.

423 16. **Komarek V**, **Maderner A**, **Spergser J**, **Weissenbock H**. 2009. Infections with weakly
424 haemolytic *Brachyspira* species in pigs with miscellaneous chronic diseases. Vet
425 Microbiol **134**:311–317.

426 17. **Jensen TK**, **Christensen AS**, **Boye M**. 2010. *Brachyspira murdochii* colitis in pigs. Vet
427 Pathol **47**:334–338.

428 18. **Mirajkar NS**, **Gebhart CJ**. 2014. Understanding the molecular epidemiology and global
429 relationships of *Brachyspira hyodysenteriae* from swine herds in the United States: A
430 multi-locus sequence typing approach. PLoS One **9**:e107176.

431 19. **Mahu M**, **de Jong E**, **De Pauw N**, **Vande Maele L**, **Vandenbroucke V**, **Vandersmissen**
432 **T**, **Miry C**, **Pasmans F**, **Haesebrouck F**, **Martel A**, **Boyen F**. 2014. First isolation of
433 *"Brachyspira hampsonii"* from pigs in Europe. Vet Rec **174**:47.2–47.

434 20. **Rohde J**, **Habighorst-Blome K**, **Seehusen F**. 2014. "*Brachyspira hampsonii*" clade I
435 isolated from Belgian pigs imported to Germany. Vet Microbiol **168**:432–435.

436 21. **Martinez-Lobo FJ**, **Hidalgo A**, **Garcia M**, **Arguello H**, **Naharro G**, **Carvajal A**, **Rubio**
437 **P**. 2013. First identification of "*Brachyspira hampsonii*" in wild European waterfowl.
438 PLoS One **8**:e82626.

439 22. **Rubin JE**, **Harms NJ**, **Fernando C**, **Soos C**, **Detmer SE**, **Harding JCS**, **Hill JE**. 2013.
440 Isolation and Characterization of *Brachyspira* spp. including "*Brachyspira hampsonii*"
441 from Lesser Snow Geese (Chen caerulescens caerulescens) in the Canadian Arctic.
442 Microb Ecol **66**:813–822.

Accepted Manuscript Posted Online

443 23. **ATCC**. Product Sheet: *Brachyspira hampsonii* (ATCC® BAA-2464™). American Type
444 Culture Collection.

445 24. **ATCC**. Product Sheet: *Brachyspira hampsonii* (ATCC® BAA-2463™). American Type
446 Culture Collection.

447 25. **Fellström C**, **Karlsson M**, **Pettersson B**, **Zimmerman U**, **Gunnarsson A**, **Aspan A**.
448 1999. Emended descriptions of indole negative and indole positive isolates of *Brachyspira*
449 *(Serpulina) hyodysenteriae*. Vet Microbiol **70**:225–238.

450 26. **Warneke HL**, **Kinyon JM**, **Bower LP**, **Burrough ER**, **Frana TS**. 2014. Matrix-assisted
451 laser desorption ionization time-of-flight mass spectrometry for rapid identification of
452 *Brachyspira* species isolated from swine, including the newly described "*Brachyspira*
453 *hampsonii*." J Vet Diagnostic Investig **26**:635–639.

454 27. **Mirajkar NS**, **Davies PR**, **Gebhart CJ**. 2016. Antimicrobial susceptibility patterns of
455 *Brachyspira* species isolated from swine herds in the United States. J Clin Microbiol
456 **54**:2109–2119.

457 28. **Costa MO**, **Hill JE**, **Fernando C**, **Lemieux HD**, **Detmer SE**, **Rubin JE**, **Harding JCS**.
458 2014. Confirmation that "*Brachyspira hampsonii*" clade I (Canadian strain 30599) causes
459 mucohemorrhagic diarrhea and colitis in experimentally infected pigs. BMC Vet Res
460 **10**:129.

461 29. **Rubin JE**, **Costa MO**, **Hill JE**, **Kittrell HE**, **Fernando C**, **Huang Y**, **O'Connor B**,
462 **Harding JCS**. 2013. Reproduction of mucohaemorrhagic diarrhea and colitis
463 indistinguishable from swine dysentery following experimental inoculation with
464 "*Brachyspira hampsonii*" strain 30446. PLoS One **8**:e57146.

Journal of Clinical Microbiology

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Journal of Clinical Microbiology

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Journal of Clinical Microbiology

Downloaded from http://jcm.asm.org/ on December 11, 2016 by SUB NO.63745291

465 30. **Wilberts BL**, **Arruda PH**, **Kinyon JM**, **Madson DM**, **Frana TS**, **Burrough ER**. 2014.
466 Comparison of lesion severity, distribution, and colonic mucin expression in pigs with
467 acute swine dysentery following oral inoculation with "*Brachyspira hampsonii*" or
468 *Brachyspira hyodysenteriae*. Vet Pathol **51**:1096–108.

469 31. **Mirajkar NS**, **Bekele AZ**, **Chander YY**, **Gebhart CJ**. 2015. Molecular epidemiology of
470 novel pathogen "*Brachyspira hampsonii*" reveals relationships between diverse genetic
471 groups, regions, host species, and other pathogenic and commensal *Brachyspira* species. J
472 Clin Microbiol **53**:2908–2918.

473 32. **Atyeo RF**, **Stanton TB**, **Jensen NS**, **Suriyaarachichi DS**, **Hampson DJ**. 1999.
474 Differentiation of *Serpulina* species by NADH oxidase gene (nox) sequence comparisons
475 and nox-based polymerase chain reaction tests. Vet Microbiol **67**:47–60.

476 33. **Neef NA**, **Lysons RJ**, **Trott DJ**, **Hampson DJ**, **Jones PW**, **Morgan JH**. 1994.
477 Pathogenicity of porcine intestinal spirochetes in gnotobiotic pigs. Infect Immun **62**:2395–
478 2403.

479 34. **Overbeek R**, **Olson R**, **Pusch GD**, **Olsen GJ**, **Davis JJ**, **Disz T**, **Edwards RA**, **Gerdes**
480 **S**, **Parrello B**, **Shukla M**, **Vonstein V**, **Wattam AR**, **Xia F**, **Stevens R**. 2014. The SEED
481 and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST).
482 Nucleic Acids Res **42**:D206–14.

483 35. **Tatusova T**, **Ciufo S**, **Federhen S**, **Fedorov B**, **McVeigh R**, **O'Neill K**, **Tolstoy I**,
484 **Zaslavsky L**. 2015. Update on RefSeq microbial genomes resources. Nucleic Acids Res
485 **43**:D599–D605.

486 36. **Meier-Kolthoff JP**, **Auch AF**, **Klenk H-P**, **Göker M**. 2013. Genome sequence-based

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487 species delimitation with confidence intervals and improved distance functions. BMC
488 Bioinformatics **14**:60.

489 37. **Lee I**, **Kim YO**, **Park SC**, **Chun J**. 2016. OrthoANI: An improved algorithm and
490 software for calculating average nucleotide identity. Int J Syst Evol Microbiol **66**:1100–
491 1103.

492 38. **Goris J**, **Konstantinidis KT**, **Klappenbach JA**, **Coenye T**, **Vandamme P**, **Tiedje JM**.
493 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence
494 similarities. Int J Syst Evol Microbiol **57**:81–91.

495 39. **Rodriguez-R LM**, **Konstantinidis KT**. 2016. The enveomics collection : a toolbox for
496 specialized analyses of microbial genomes and metagenomes. Peer J Prepr **4**:e1900v1.

497 40. **Mende DR**, **Sunagawa S**, **Zeller G**, **Bork P**. 2013. Accurate and universal delineation of
498 prokaryotic species. Nat Methods **10**:881–884.

499 41. **Schleifer KH**. 2009. Classification of Bacteria and Archaea: Past, present and future. Syst
500 Appl Microbiol **32**:533–542.

501 42. **Tindall BJ**, **Rosselló-Móra R**, **Busse HJ**, **Ludwig W**, **Kämpfer P**. 2010. Notes on the
502 characterization of prokaryote strains for taxonomic purposes. Int J Syst Evol Microbiol
503 **60**:249–266.

504 43. **Auch AF**, **von Jan M**, **Klenk H-P**, **Göker M**. 2010. Digital DNA-DNA hybridization for
505 microbial species delineation by means of genome-to-genome sequence comparison.
506 Stand Genomic Sci **2**:117–34.

507 44. **Håfström T**, **Jansson DS**, **Segerman B**. 2011. Complete genome sequence of

Journal of Clinical Microbiology

Accepted Manuscript Posted Online

Journal of Clinical Microbiology

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508 *Brachyspira intermedia* reveals unique genomic features in *Brachyspira* species and
509 phage-mediated horizontal gene transfer. BMC Genomics **12**:395.

510 45. **Bellgard MI**, **Wanchanthuek P**, **La T**, **Ryan K**, **Moolhuijzen P**, **Albertyn Z**, **Shaban**
511 **B**, **Motro Y**, **Dunn DS**, **Schibeci D**, **Hunter A**, **Barrero R**, **Phillips ND**, **Hampson DJ**.
512 2009. Genome sequence of the pathogenic intestinal spirochete *Brachyspira*
513 *hyodysenteriae* reveals adaptations to its lifestyle in the porcine large intestine. PLoS One
514 **4**:e4641–e4641.

515 46. **Wanchanthuek P**, **Bellgard M**, **La T**, **Ryan K**, **Moolhuijzen P**, **Chapman B**, **Black M**,
516 **Schibeci D**, **Hunter A**, **Barrero R**, **Phillips N**, **Hampson D**. 2010. The complete genome
517 sequence of the pathogenic intestinal spirochete *Brachyspira pilosicoli* and comparison
518 with other *Brachyspira* genomes. PLoS One **5**:e11455–e11455.

519 47. **Pati A**, **Sikorski J**, **Gronow S**, **Munk C**, **Lapidus A**, **Copeland A**, **Glavina Del Tio T**,
520 **Nolan M**, **Lucas S**, **Chen F**, **Tice H**, **Cheng J-F**, **Han C**, **Detter JC**, **Bruce D**, **Tapia R**,
521 **Goodwin L**, **Pitluck S**, **Liolios K**, **Ivanova N**, **Mavromatis K**, **Mikhailova N**, **Chen A**,
522 **Palaniappan K**, **Land M**, **Hauser L**, **Chang Y-J**, **Jeffries CD**, **Spring S**, **Rohde M**,
523 **Göker M**, **Bristow J**, **Eisen JA**, **Markowitz V**, **Hugenholtz P**, **Kyrpides NC**, **Klenk H-**
524 **P**. 2010. Complete genome sequence of *Brachyspira murdochii* type strain (56-150T).
525 Stand Genomic Sci **2**:260–269.

526 48. **Wayne LG**, **Brenner DJ**, **Colwell RR**, **Grimont P a. D**, **Kandler O**, **Krichevsky MI**,
527 **Moore LH**, **Moore WEC**, **Murray RGE**, **Stackebrandt E**, **Starr MP**, **Truper HG**.
528 1987. Report of the ad hoc committee on reconciliation of approaches to bacterial
529 systematics. Int J Syst Bacteriol **37**:463–464.

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530 49. **Richter M**, **Rosselló-Móra R**. 2009. Shifting the genomic gold standard for the
531 prokaryotic species definition. Proc Natl Acad Sci U S A **106**:19126–31.

532 50. **Konstantinidis KT**, **Tiedje JM**. 2005. Towards a genome-based taxonomy for
533 prokaryotes. J Bacteriol **187**:6258–6264.

534 51. **Ursing JB**, **Rossellomora RA**, **Garciavaldes E**, **Lalucat J**. 1995. Taxonomic note - A
535 pragmatic approach to the nomenclature of phenotypically similar genomic groups. Int J
536 Syst Bacteriol **45**:604.

537

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538 **TABLES**

539 **Table 1: Comparison of mean cell dimensions of *B. hampsonii* strains**

	NSH-16[a]		**NSH-24**[a]		**P280/1**[a]	
	Length	Width	Length	Width	Length	Width
Mean	5.43	0.34	5.06	0.28	10.49	0.33
SD[b]	0.43	0.01	0.37	0.03	0.41	0.01

540 Legend for Table 1:

541 [a]Measurements in µm

542 [b]SD, standard deviation

543

544 **Table 2: Genome Assembly Statistics and Annotation Features of *B. hampsonii* strains**

Genome Features	**NSH-16** [T]	**NSH-24**	**P280/1**
Genome status	Draft	Draft	Draft
Total Assembly size	3,161,271 bp	2,969,002 bp	3,186,631 bp
Number of contigs	77	178	16
N50	88,495 bp	29,547 bp	690,165
L50	13	29	2
G+C content	27.4%	27.5%	27.5%
Number of subsystems	309	307	309
Number of coding sequences	2822	2576	2945
Number of predicted RNAs	36	35	39

545

546

547 **Table 32: Genome to Genome Distance comparisons of *B. hampsonii* and other valid**

548 ***Brachyspira* species**

Reference genome	**Genome to Genome Distance values [Model C.I.]**[a]		
	B. hampsonii NSH-16 [T]	*B. hampsonii* NSH-24	*B. hampsonii* P280/1

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B. hampsonii NSH-16 [T]	100 [100 - 100%]	50.5 [47.9 – 53.2%]	53.2 [50.5 – 55.9%]
B. hampsonii 30599	98.8 [98.2 - 99.2%]	51.3 [48.7 - 54%]	53.9 [51.2 – 56.5%]
B. hampsonii NSH-24	50.5 [47.9 – 53.2%]	100 [100 - 100%]	57.2 [54.4 – 59.9%]
B. hampsonii 30446	50.2 [47.6 – 52.9%]	99.6 [99.3 - 99.8%]	56.9 [54.1 – 59.7%]
B. hampsonii P280/1	53.2 [50.5 – 55.9%]	57.2 [54.4 – 59.9%]	100 [100 - 100%]
B. hyodysenteriae B-78 [T]	34.6 [32.2 – 37.2%]	34 [31.5 – 36.5%]	34.2 [31.8 – 36.7%]
B. suanatina AN4859/03 [T]	34.7 [32.2 – 37.2%]	34.1 [31.7 – 36.6%]	34.3 [31.9 – 36.8%]
B. intermedia PWS/A [T]	35 [37.2 - 42.3%]	34.4 [31.9 – 36.9%]	34.6 [32.2 – 37.1%]
B. murdochii 56-150 [T]	30.2 [27.8 – 32.7%]	29.6 [27.2 – 32.1%]	30.2 [27.8 – 32.7%]
B. innocens B256 [T]	29.7 [27.3 – 32.2%]	29.5 [27.1 - 32%]	29.8 [27.4 – 32.3%]
B. alvinipulli 911207/C1 [T]	25.8 [23.5 – 28.3%]	25.6 [23.3 – 28.1%]	25.6 [23.2 – 28%]
B. pilosicoli P43/6/78 [T]	24.9 [22.6 – 27.4%]	24.7 [22.3 – 27.1%]	24.9 [22.6 – 27.4%]
B. aalborgii 513A [T]	20.9 [18.6 – 23.3%]	20.2 [18 – 22.6%]	21.1 [18.8 – 23.5%]

549 Legend for Table 3:

550 [a] GGD values have been calculated using the recommended Formula 2 as it is independent of the

551 length of genomes, and thus robust against the use of draft genomes.

552

553 **Table 4: Average Nucleotide Identity of *B. hampsonii* and all valid *Brachyspira* species**

554

555

556

557

558

559

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Reference genome	**ANI values**		
	B. hampsonii NSH-16 [T]	*B. hampsonii* NSH-24	*B. hampsonii* P280/1
B. hampsonii NSH-16 [T]	100%	93.39%	93.70%
B. hampsonii 30599	99.83%	93.33%	93.72%
B. hampsonii NSH-24	92.82%	100%	94.50%
B. hampsonii 30446	92.82%	99.9%	94.44%
B. hampsonii P280/1	93.52%	94.52%	100%
B. hyodysenteriae B-78 [T]	88%	87.63%	87.83%
B. suanatina AN4859/03 [T]	88.06%	87.7%	87.96%
B. intermedia PWS/A [T]	88.19%	87.8%	88.01%
B. murdochii 56-150 [T]	84.71%	84.3%	84.74%
B. innocens B256 [T]	84.35%	84.5%	84.59%
B. alvinipulli 911207/C1 [T]	82.07%	81.75%	81.86%
B. pilosicoli P43/6/78 [T]	78.26%	78.25%	78.16%
B. aalborgii 513A [T]	74.88%	74.91%	74.78%

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562 **Table 5: Average Amino Acid Identity of *B. hampsonii* and all valid *Brachyspira* species**

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Reference genome	AAI values		
	B. hampsonii NSH-16 T	*B. hampsonii* NSH-24	*B. hampsonii* P280/1
B. hampsonii NSH-16 T	100%	94.07%	94.09%
B. hampsonii 30599	99.72%	94.03%	94.16%
B. hampsonii NSH-24	94.09%	100%	95.04%
B. hampsonii 30446	94.07%	99.95%	95.12%
B. hampsonii P280/1	94.08%	95.04%	100%
B. hyodysenteriae B-78 T	89.59%	89.11%	89.06%
B. suanatina AN4859/03 T	89.27%	88.92%	88.88%
B. intermedia PWS/A T	89.58%	88.95%	88.94%
B. murdochii 56-150 T	84.87%	84.90%	85.42%
B. innocens B256 T	84.41%	84.78%	84.55%
B. alvinipulli 911207/C1 T	80.98%	80.61%	80.50%
B. pilosicoli P43/6/78 T	75.04%	75.05%	74.96%
B. aalborgii 513A T	71.57%	71.62%	71.55%

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571 **Table 6: Comparison of *B. hampsonii* to known valid bacterial genomes using SpecI**

Query genome	Result	Closest match	
		NCBI Taxonomy name	Average % identity
B. hampsonii NSH-16 (genomovar I)	Could not be assigned a species	*Brachyspira hyodysenteriae* WA1	93.31%
B. hampsonii NSH-24 (genomovar II)	Could not be assigned a species	*Brachyspira intermedia* PWS/A	92.92%
B. hampsonii P280/1 (genomovar III)	Could not be assigned a species	*Brachyspira intermedia* PWS/A	93.34%

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574 **FIGURE LEGENDS**

575 **Figure 1: Phase contrast micrograph of *B. hampsonii* strain NSH-24 cells viewed at 100X**

576 **showing one to two flat serpentine coils and slightly tapered ends.**

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578 **Figure 2: Electron micrograph of negatively stained *B. hampsonii* strain NSH-16^{T} showing**

579 **12 periplasmic flagella at one end of the cell.** The cell was viewed at 60,000X magnification

580 and the scale bar represents 500 nm.

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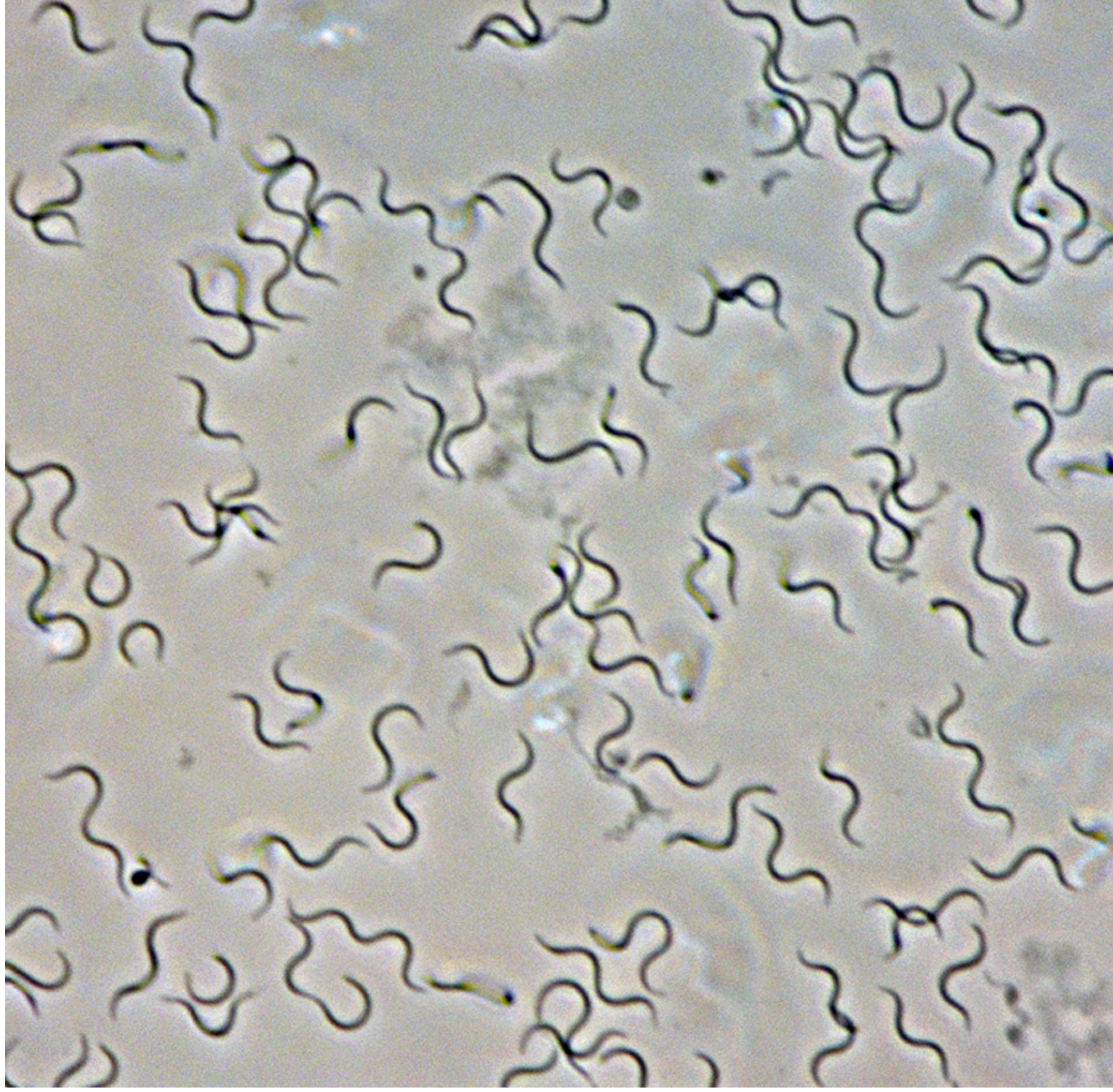

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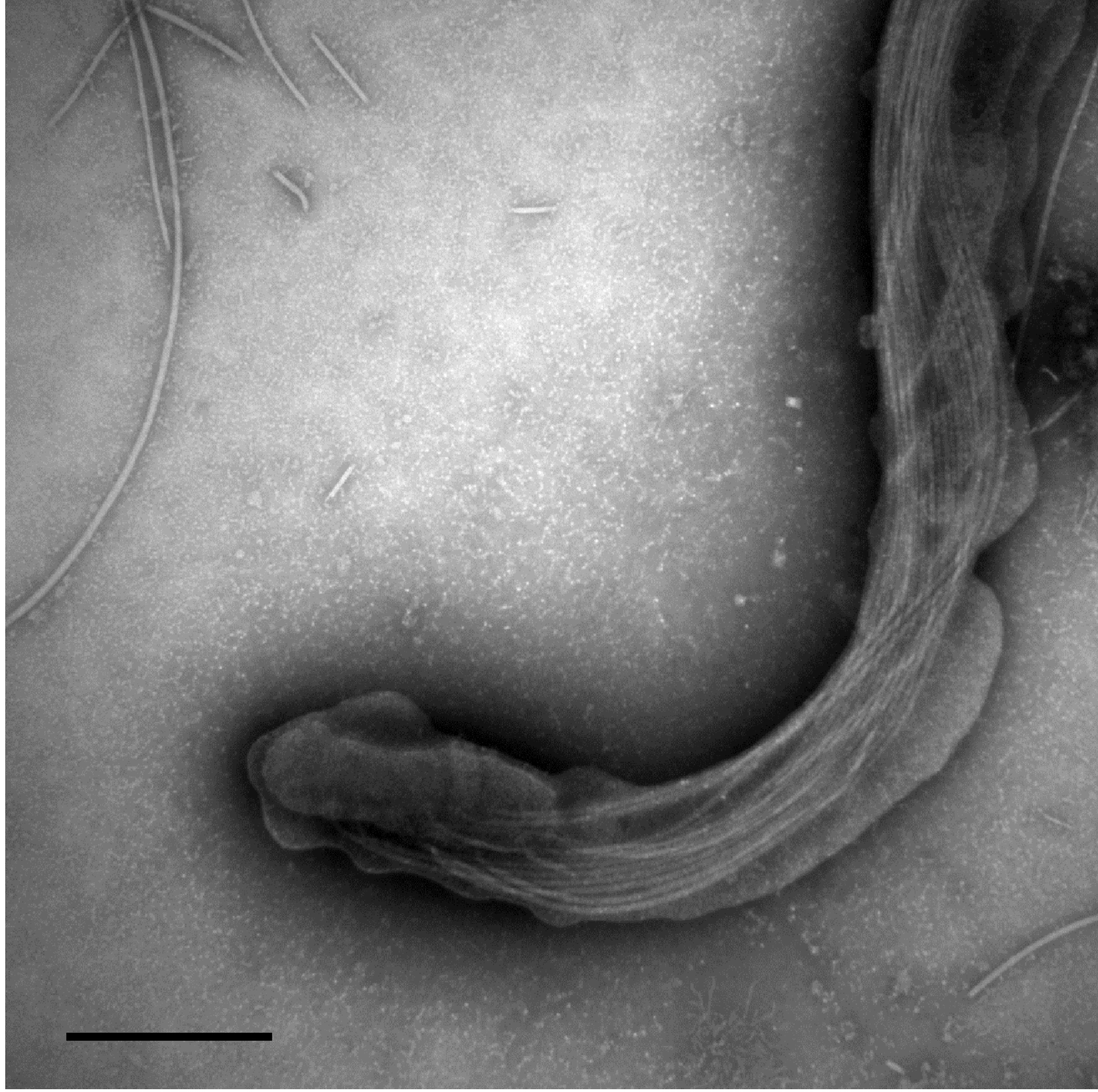

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