Reclassification of *Serpulina intermedia* and *Serpulina murdochii* in the genus *Brachyspira* as *Brachyspira intermedia* comb. nov. and *Brachyspira murdochii* comb. nov.

David J. Hampson and Tom La

Correspondence

David J. Hampson
d.hampson@murdoch.edu.au

School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, Western Australia 6150, Australia

A reassessment was undertaken of published characteristics of the five species of anaerobic intestinal spirochaetes in the genus *Brachyspira* (*Brachyspira aalborgi*, *Brachyspira alvinipulli*, *Brachyspira hyodysenteriae*, *Brachyspira innocens* and *Brachyspira pilosicoli*) and the two species in the genus *Serpulina* (*Serpulina intermedia* and *Serpulina murdochii*). Comparisons were made of published descriptions, including phenotypic properties, grouping by multilocus enzyme electrophoresis, DNA base composition, DNA–DNA relative reassociation values and 16S rRNA gene sequence similarity. On the basis of extensive similarities between all the species, it is proposed to reclassify *Serpulina intermedia* Stanton *et al.* 1997 and *Serpulina murdochii* Stanton *et al.* 1997 in the genus *Brachyspira*, as *Brachyspira intermedia* comb. nov. (type strain ATCC 51140^T) and *Brachyspira murdochii* comb. nov. (type strain ATCC 51284^T).

Anaerobic spirochaetes colonize the distal intestinal tracts of human beings and various species of animals and birds (Hampson & Stanton, 1997). Currently, the two main genera of clinical interest are *Brachyspira* and *Serpulina*, as they include pathogenic as well as commensal species. The genus *Brachyspira*, originally containing the single species *Brachyspira aalborgi*, was proposed in 1982 (Hovind-Hougen *et al.*, 1982). *B. aalborgi* colonizes humans and its pathogenicity remains uncertain. The genus *Serpulina*, containing the species *Serpulina hyodysenteriae* and *Serpulina innocens*, was proposed by Stanton (1992). These two species colonize pigs and were originally allocated to the genus *Treponema* as *Treponema hyodysenteriae*, the agent of swine dysentery (Harris *et al.*, 1972), and the non-pathogenic *Treponema innocens* (Kinyon & Harris, 1979). Subsequently, both species were transferred briefly to the proposed new genus *Serpula* (Stanton *et al.*, 1991). Because this genus name was discovered to be illegitimate due to the prior existence of the fungal genus *Serpula*, both species were then reclassified in the new genus *Serpulina*. A third *Serpulina* species, *Serpulina pilosicoli*, was proposed in 1996 (Trott *et al.*, 1996b). *S. pilosicoli* was pathogenic to pigs and chickens and colonized humans and many species of animals and birds. Subsequently, it was proposed that the

genera *Serpulina* and *Brachyspira* be unified and that *S. hyodysenteriae*, *S. innocens* and *S. pilosicoli* be transferred to the genus *Brachyspira*, as *Brachyspira hyodysenteriae* comb. nov., *Brachyspira innocens* comb. nov. and *Brachyspira pilosicoli* comb. nov. (Ochiai *et al.*, 1997). These new name combinations appeared in Validation List no. 64 (Ochiai *et al.*, 1998). Concurrently, a description of the novel species *Serpulina alvinipulli* was published, with the updated name *Brachyspira alvinipulli* added in proof in response to the transfer of the three other former *Serpulina* species to the genus *Brachyspira* (Stanton *et al.*, 1998). Two further *Serpulina* species, *Serpulina intermedia* and *Serpulina murdochii*, were proposed in 1997 (Stanton *et al.*, 1997). Unfortunately, due to the timing of their description, they were not included in the transfer of species when the genera *Serpulina* and *Brachyspira* were unified in 1997. This situation has since been a source of some confusion, with various authors now pragmatically electing to call the two remaining *Serpulina* species ‘*Brachyspira intermedia*’ and ‘*Brachyspira murdochii*’, respectively, rather than the validly published names *S. intermedia* and *S. murdochii*.

The current paper proposes the transfer of *S. intermedia* and *S. murdochii* to the genus *Brachyspira*. In support of this proposal, published data relating to the five current *Brachyspira* species and the two *Serpulina* species (Stanton *et al.*, 1991, 1996, 1997, 1998; Ochiai *et al.*, 1997) were reassessed. All seven species are slow-growing anaerobic spirochaetes that colonize the distal intestinal tract. All seven have a low G + C content, with values for the type strains of the

Abbreviation: MLEE, multilocus enzyme electrophoresis.

The GenBank/EMBL/DDJB accession numbers for the 16S rRNA gene sequences of *Brachyspira intermedia* ATCC 51140^T and *Brachyspira murdochii* ATCC 51284^T are U23033 and AY312492, respectively.

species varying in the range 24.6 mol% for *B. alvinipulli* ATCC 51933^T (Stanton *et al.*, 1998) to 27.1 mol% for *B. aalborgi* NTCC 11492^T (Ochiai *et al.*, 1997). *S. intermedia* ATCC 51140^T has a G+C content of 25 mol% and *S. murdochii* ATCC 51284^T a G+C content of 27 mol% (Stanton *et al.*, 1997). In a study using multilocus enzyme electrophoresis (MLEE) and partial sequencing of the 16S rRNA gene to analyse strains of the seven intestinal spirochaete species, these species were clustered together, with *B. aalborgi* being the most distantly related of the species (Stanton *et al.*, 1996). These results are consistent with the two *Serpulina* species belonging to the same genus as the *Brachyspira* species. To illustrate these relationships, partial (1205 bp) 16S rRNA gene sequences of the seven type strains were obtained from the DDBJ/GenBank/EMBL databases and used to create a dendrogram (Fig. 1). Again, *B. aalborgi* appears as the outlying species. Pairwise alignments of these sequences for the seven type strains gave similarity values in the range 95.5–99.7 %, consistent with the species belonging to the same genus (Ochiai *et al.*, 1997). *S. intermedia* ATCC 51140^T was most closely related to *B. hyodysenteriae* ATCC 27164^T (99.7 % similarity), whereas *S. murdochii* was most closely related to *B. innocens* ATCC 29796^T (99.4 % similarity).

If the seven species belong to the same genus, the question then arises as to whether the two remaining *Serpulina* species are distinct from the current five *Brachyspira* species. Arguments in support of separate species identity were made at the time that *S. intermedia* and *S. murdochii* were described (Stanton *et al.*, 1997) and are reiterated. *B. hyodysenteriae* is the species most closely related to *S. intermedia*. Besides their almost identical 16S rRNA gene sequences, DNA from the type strains of *B. hyodysenteriae* and *S. intermedia* showed 57–68 % relative reassociation (Stanton *et al.*, 1997), closely approaching the value of 70 % that is usually used to indicate that two strains belong to the

same species (Wayne *et al.*, 1987). Furthermore, they share a number of phenotypic properties: both produce indole and typically have α -glucosidase and β -glucosidase activity when tested in the commercial API ZYM system (Lee *et al.*, 1993; Fellström & Gunnarsson, 1995). However, the two species can be distinguished by MLEE analysis (Stanton *et al.*, 1996) and on the basis of consistent differences in the sequences of their 23S rRNA genes (Leser *et al.*, 1997) and their NADH oxidase genes (Atyeo *et al.*, 1999). A notable feature of *B. hyodysenteriae* is that it produces a strong β -haemolysis on blood agar, whereas *S. intermedia* produces weak haemolysis. Infection of the porcine colon with *B. hyodysenteriae* induces a severe mucohaemorrhagic diarrhoea (swine dysentery), whereas *S. intermedia* is generally not considered to be pathogenic in pigs (Harris *et al.*, 1999). Hence the two species have clear phenotypic and biological differences that can be used to differentiate them.

The second *Serpulina* species, *S. murdochii*, is most closely related to *B. innocens*. These two species have very similar 16S rRNA gene sequences and the type strains have 64–66 % DNA–DNA relative reassociation (Stanton *et al.*, 1997), which again is borderline in terms of differentiating species (Wayne *et al.*, 1987). However, they clearly cluster in different groups in MLEE analysis (Stanton *et al.*, 1996; McLaren *et al.*, 1997) and can be separated on the basis of differences in their NADH oxidase gene sequences (Atyeo *et al.*, 1999) and by restriction fragment length polymorphism analysis of PCR-generated 16S rRNA gene products (Stanton *et al.*, 1998). They also have minor phenotypic differences, with *B. innocens* typically being α -galactosidase-positive whereas *S. murdochii* is typically negative for this activity (Lee *et al.*, 1993; Fellström & Gunnarsson, 1995). These differences are sufficient to allow them to be distinguished as separate species.

Taken together, these data demonstrate that the seven named species of anaerobic intestinal spirochaetes are

Fig. 1. Unrooted phylogenetic tree of the genus *Brachyspira* and related proposed species *B. intermedia* comb. nov. and *B. murdochii* comb. nov. The tree was generated using the neighbour-joining method (Saitou & Nei, 1987) based on partial (1205 bp) 16S rRNA gene sequences retrieved from the DDBJ/GenBank/EMBL databases (accession numbers are given in parentheses). Phylogenetic distance values were generated using MEGA version 3.0 (Kumar *et al.*, 2004) and the Jukes–Cantor model was used to account for nucleotide substitutions. Bar, relative sequence divergence (5 estimated substitutions per 1000 base positions).

distinct, but are closely related, and that it is appropriate that they be unified in a single genus. The genus name *Brachyspira* has chronological precedence over the name *Serpulina* and hence all seven species should be located in the genus *Brachyspira*. Therefore, we propose the transfer of *Serpulina intermedia* and *Serpulina murdochii* to the genus *Brachyspira*, as *Brachyspira intermedia* comb. nov. (type strain ATCC 51140^T) and *Brachyspira murdochii* comb. nov. (type strain ATCC 51284^T).

Description of *Brachyspira intermedia* comb. nov.

Brachyspira intermedia [in.ter.me'di.a. L. fem. adj. *intermedia* that is between, intermediate, referring to the fact that the biochemical reactivities of this organism are intermediate between those characteristically possessed by *B. hyodysenteriae* and by *B. innocens* (Lee *et al.*, 1993)].

Basonym: *Serpulina intermedia* Stanton *et al.* 1997.

Previously also known as '*Serpulina intermedius*' (Lee *et al.*, 1993). Some intestinal spirochaetes referred to as '*Treponema hyodysenteriae*' biotype 2 or 'intermediate type' may be *B. intermedia* strains (Binek & Szykiewicz, 1984).

B. intermedia strains have been isolated from commercial poultry flocks exhibiting diarrhoea and reduced production (McLaren *et al.*, 1997; Stephens *et al.*, 2005), and experimental infection of laying hens with a pure culture of a *B. intermedia* strain has caused reduced egg production and increased faecal water content (Hampson & McLaren, 1999). The description is as given previously by Stanton *et al.* (1997).

The type strain is ATCC 51140^T (= CIP 105833^T).

Description of *Brachyspira murdochii* comb. nov.

Brachyspira murdochii (mur.do'chi.i. N.L. masc. gen. n. *murdochii* of Murdoch, in recognition of work conducted at Murdoch University in Western Australia, where the type strain was identified).

Basonym: *Serpulina murdochii* Stanton *et al.* 1997.

Previously known as 'group B spirochaetes' and *S. murdochii* (Lee *et al.*, 1993; Lee & Hampson, 1994). *B. murdochii* strains have been isolated from the large intestinal contents of healthy swine and rats (Trott *et al.*, 1996a) and from chickens (Stephens *et al.*, 2005). The spirochaete generally is not considered to be a pathogen, although occasionally it has been seen in association with colitis in pigs (Weissenböck *et al.*, 2005). The description is as given previously by Stanton *et al.* (1997).

The type strain is ATCC 51284^T (= CIP 105832^T = DSM 12563^T).

References

- Atyeo, R. F., Stanton, T. B., Jensen, N. S., Suriyaarachichi, D. S. & Hampson, D. J. (1999). Differentiation of *Serpulina* species by NADH oxidase gene (*nox*) sequence comparisons and *nox*-based polymerase chain reaction tests. *Vet Microbiol* **67**, 47–60.
- Binek, M. & Szykiewicz, Z. M. (1984). Physiological properties and classification of strains of *Treponema* sp. isolated from pigs in Poland. *Comp Immunol Microbiol Infect Dis* **7**, 141–148.
- Fellström, C. & Gunnarsson, A. (1995). Phenotypical characterisation of intestinal spirochaetes isolated from pigs. *Res Vet Sci* **59**, 1–4.
- Hampson, D. J. & McLaren, A. J. (1999). Experimental infection of layer hens with *Serpulina intermedia* causes reduced egg production and increased faecal water content. *Avian Pathol* **28**, 113–117.
- Hampson, D. J. & Stanton, T. B. (1997). *Intestinal Spirochaetes in Domestic Animals and Humans*. Wallingford, UK: CAB International.
- Harris, D. L., Glock, R. D., Christensen, C. R. & Kinyon, J. M. (1972). Inoculation of pigs with *Treponema hyodysenteriae* (new species) and reproduction of the disease. *Vet Med Small Anim Clin* **67**, 61–64.
- Harris, D. L., Hampson, D. J. & Glock, R. (1999). Swine dysentery. In *Diseases of Swine*, 8th edn, pp. 579–600. Edited by B. E. Straw, S. D'Allaire, W. L. Mengeling & D. J. Taylor. Ames, IA: Iowa State University Press.
- Hovind-Hougen, K., Birch-Andersen, A., Hendrik-Nielsen, R., Orholm, M., Pedersen, J. O., Teglbjærg, P. S. & Thaysen, E. H. (1982). Intestinal spirochetosis: morphological characterization and cultivation of the spirochete *Brachyspira aalborgi* gen. nov., sp. nov. *J Clin Microbiol* **16**, 1127–1136.
- Kinyon, J. M. & Harris, D. L. (1979). *Treponema innocens*, a new species of intestinal bacteria, and emended description of the type strain of *Treponema hyodysenteriae* Harris *et al.* *Int J Syst Bacteriol* **29**, 102–109.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Lee, J. I. & Hampson, D. J. (1994). Genetic characterisation of intestinal spirochaetes and their association with disease. *J Med Microbiol* **40**, 365–371.
- Lee, J. I., Hampson, D. J., Lymbery, A. J. & Harders, S. J. (1993). The porcine intestinal spirochaetes: identification of new genetic groups. *Vet Microbiol* **34**, 273–285.
- Leser, T. D., Möller, M., Jensen, T. K. & Jorsal, S. E. (1997). Specific detection of *Serpulina hyodysenteriae* and potentially pathogenic weakly β -haemolytic porcine intestinal spirochetes by polymerase chain reaction targeting 23S rDNA. *Mol Cell Probes* **11**, 363–372.
- McLaren, A. J., Trott, D. J., Swayne, D. E., Oxberry, S. L. & Hampson, D. J. (1997). Genetic and phenotypic characterization of intestinal spirochetes colonizing chickens and allocation of known pathogenic isolates to three distinct genetic groups. *J Clin Microbiol* **35**, 412–417.
- Ochiai, S., Adachi, Y. & Mori, K. (1997). Unification of the genera *Serpulina* and *Brachyspira*, and proposals of *Brachyspira hyodysenteriae* comb. nov., *Brachyspira innocens* comb. nov. and *Brachyspira pilosicoli* comb. nov. *Microbiol Immunol* **41**, 445–452.
- Ochiai, S., Adachi, Y. & Mori, K. (1998). *Brachyspira hyodysenteriae* comb. nov., *Brachyspira innocens* comb. nov. and *Brachyspira pilosicoli* comb. nov. In *Validation of Publication of New Names and New Combinations Previously Effectively Published Outside the IJSEM*, List no. 64. *Int J Syst Bacteriol* **48**, 327–328.

- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Stanton, T. B. (1992). Proposal to change the genus designation *Serpula* to *Serpulina* gen. nov. containing the species *Serpulina hyodysenteriae* comb. nov. and *Serpulina innocens* comb. nov. *Int J Syst Bacteriol* **42**, 189–190.
- Stanton, T. B., Jensen, N. S., Casey, T. A., Tordoff, L. A., Dewhirst, F. E. & Paster, B. J. (1991). Reclassification of *Treponema hyodysenteriae* and *Treponema innocens* in a new genus, *Serpula* gen. nov., as *Serpula hyodysenteriae* comb. nov. and *Serpula innocens* comb. nov. *Int J Syst Bacteriol* **41**, 50–58.
- Stanton, T. B., Trott, D. J., Lee, J. I., McLaren, A. J., Hampson, D. J., Paster, B. J. & Jensen, N. S. (1996). Differentiation of intestinal spirochaetes by multilocus enzyme electrophoresis analysis and 16S rRNA sequence comparisons. *FEMS Microbiol Lett* **136**, 181–186.
- Stanton, T. B., Fournié-Amazouz, E., Postic, D., Trott, D. J., Grimont, P. A. D., Baranton, G., Hampson, D. J. & Saint Girons, I. (1997). Recognition of two new species of intestinal spirochetes: *Serpulina intermedia* sp. nov. and *Serpulina murdochii* sp. nov. *Int J Syst Bacteriol* **47**, 1007–1012.
- Stanton, T. B., Postic, D. & Jensen, N. S. (1998). *Serpulina alvinipulli* sp. nov., a new *Serpulina* species that is enteropathogenic for chickens. *Int J Syst Bacteriol* **48**, 669–676.
- Stephens, C. P., Oxberry, S. L., Phillips, N. D., La, T. & Hampson, D. J. (2005). The use of multilocus enzyme electrophoresis to characterise intestinal spirochaetes (*Brachyspira* spp.) colonising hens in commercial flocks. *Vet Microbiol* **107**, 149–157.
- Trott, D. J., Atyeo, R. F., Lee, J. I., Swayne, D. E., Stoutenburg, J. W. & Hampson, D. J. (1996a). Genetic relatedness amongst intestinal spirochaetes isolated from rats and birds. *Lett Appl Microbiol* **23**, 431–436.
- Trott, D. J., Stanton, T. B., Jensen, N. S., Duhamel, G. E., Johnson, J. L. & Hampson, D. J. (1996b). *Serpulina pilosicoli* sp. nov., the agent of porcine intestinal spirochetosis. *Int J Syst Bacteriol* **46**, 206–215.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Weissenböck, H., Maderner, A., Herzog, A. M., Lussy, H. & Nowotny, N. (2005). Amplification and sequencing of *Brachyspira* spp. specific portions of *nox* using paraffin-embedded tissue samples from clinical colitis in Austrian pigs shows frequent solitary presence of *Brachyspira murdochii*. *Vet Microbiol* **111**, 67–75.