Reclassification of *Leptospira parva* Hovind-Hougen *et al.* 1982 as *Turneriella parva* gen. nov., comb. nov.

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Analysis of the G + C content, DNA–DNA relatedness to other leptospires and 16S rRNA gene sequence of *Leptospira parva* showed that this species was not related to other *Leptospira* species. On the basis of these data, it is proposed that *Leptospira parva* should be transferred to the genus *Turneriella* as *Turneriella parva* gen. nov., comb. nov., with strain H^T (=NCTC 11395^T = ATCC BAA-1111^T) as the type strain.

Leptospires were traditionally classified as belonging to two species, *Leptospira interrogans* and *Leptospira biflexa*, comprising pathogenic and non-pathogenic strains, respectively (Johnson & Faine, 1984). A more complex classification, based upon DNA–DNA relatedness, recognizes 13 species with validly published names and four as yet unnamed genomospecies (Brenner *et al.*, 1999; Perolat *et al.*, 1998; Ramadass *et al.*, 1992; Yasuda *et al.*, 1987).

Leptospira parva strain H^T was isolated from contaminated bovine albumin liquid culture medium, and was recognized as being distinct from strains belonging to both existing species of the genus *Leptospira* (Hovind-Hougen *et al.*, 1981). Moreover, the G + C content differed markedly from that of strains of other *Leptospira* species (Hovind-Hougen *et al.*, 1981). That *Leptospira parva* was not closely related to other species within the genus was further confirmed by DNA–DNA relatedness studies (Yasuda *et al.*, 1987) and 16S rRNA gene sequencing (Hookey *et al.*, 1993) (Fig. 1).

A second strain, S-308-81 (=ATCC BAA-1112), was isolated from the uterus of a sow in Northern Ireland. The 16S rRNA gene sequence of this strain was found to be identical to that of strain H^T. DNA was extracted from strains H^T and S-308-81 and purified, as described previously (Brenner *et al.*, 1982). DNA from strain H^T was labelled with [³²P]dCTP (Brenner *et al.*, 1999), and the DNA–DNA relatedness and percentage divergence between the two strains

were determined by using the hydroxyapatite method, at an optimal reassociation temperature of 55 °C. The DNA–DNA relatedness (relative binding ratio) was 81 % and the percentage divergence in related DNA sequences was 0.2 % (Table 1). These two strains meet the criteria for the molecular definition of a species (Brenner *et al.*, 1999). DNA–DNA relatedness studies (Table 1) have shown *Leptospira parva* strain H^T to be distinct from strains of other *Leptospira* species and from *Leptonema illini* (Brenner *et al.*, 1999).

A proposal was made at the ICSB Subcommittee on the Taxonomy of *Leptospira* (TSC) meeting in 1990 to transfer *Leptospira parva* from the genus *Leptospira* to a newly created genus, 'Turneria', as 'Turneria parva' (Marshall, 1992). This proposal was agreed to at the TSC meeting, but apparently was not prepared for publication, so the name currently has no standing in nomenclature. The nomenclature of this species has become a source of confusion, with some authors referring incorrectly to *Leptospira parva* as *Leptospira parva incertae sedis* (Hookey, 1993) and others using the genus name 'Turneria', which has no standing in nomenclature. Moreover, the genus name 'Turneria' is illegitimate because it is the name of genera of both plants (Dicotyledones: Turneraceae) and animals (Formicidae, Hymenoptera and Sauropterygia, Reptilia).

In order to clarify this situation, we propose the transfer of *Leptospira parva* to the genus *Turneriella* as *Turneriella parva* gen. nov., comb. nov., with strain H^T as the type strain (Hovind-Hougen *et al.*, 1981). We believe that this fulfils the original intention of the proposal minuted from the 1990 TSC meeting (Marshall, 1992). The description of *T. parva* is given below, and the description of the family *Leptospiaceae* Hovind-Hougen 1979 is emended, to take into account the proposed changes.

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains H^T and S-308-81 are AY293856 and AY398688, respectively.

Fig. 1. Phylogenetic relationships between the genera of the family *Leptospiraceae*. The unrooted tree was obtained by neighbour-joining 16S rRNA gene sequences representing the leptospiral clades, *Leptonema illini* and *T. parva*. The 16S rRNA genes of strains of known species were resequenced to near full-length and high redundancy before further analysis. Neighbour-joining was implemented on CLUSTAL_X-aligned sequences, with branch placement analysed by bootstrapping 1000 times for confidence resulting in all branch values of 1000 (not shown). The tree was drawn using TreeExplorer. Bar, 29 nucleotide substitutions per 1454 aligned base pairs. GenBank/EMBL/DDBJ accession numbers are as follows. Pathogenic *Leptospira* spp.: *Leptospira borgpetersenii* serovar Ballum strain Mus 127, AY631884. Intermediate *Leptospira* spp.: *Leptospira inadaei* serovar Lyme^T, AY631896; *Leptospira fainei* serovar Hurstbridge strain BUT 6^T, AY631885. Saprophytic *Leptospira* species: *Leptospira biflexa* serovar Patoc strain Patoc I^T, AY631876. *Leptonema illini* serovar Illini strain 3055^T, AY714984. *T. parva* strain H^T, AY293856.

Table 1. DNA–DNA relatedness between *T. parva* H^T and reference strains of *Leptospira* species and *Leptonema illini*

Source of unlabelled DNA	Relatedness to labelled DNA from <i>T. parva</i> H ^T (%)	
	55 °C	70 °C
<i>T. parva</i> H ^T	100*	ND
<i>T. parva</i> S-308-81	81*	ND
<i>Leptospira alexanderi</i> L 60 ^T	ND	0
<i>Leptospira biflexa</i> CH11	0	2
<i>Leptospira borgpetersenii</i> Veldrat Bataviae 46 ^T	2	1
<i>Leptospira fainei</i> BUT 6 ^T	8*	ND
<i>Leptospira inadaei</i> 10 ^T	7	0
<i>Leptospira interrogans</i> RGA ^T	0	2
<i>Leptospira kirschneri</i> 3522C ^T	1	3
<i>Leptospira meyeri</i> Iowa City Frog ^T	ND	1
<i>Leptospira noguchii</i> CZ 214 ^T	1	3
<i>Leptospira santarosai</i> 1342 K	1	ND
<i>Leptospira weilii</i> Celledoni ^T	3	7
<i>Leptospira wolbachii</i> CDC ^T	ND	1
<i>Leptospira</i> genomospecies 1 80-412	2	1
<i>Leptospira</i> genomospecies 3 WaZ Holland ^T	8	0
<i>Leptospira</i> genomospecies 4 LT 11-33	2	1
<i>Leptospira</i> genomospecies 5 Sao Paulo ^T	1	ND
<i>Leptonema illini</i> 3055 ^T	ND	0

ND, Not done.

*Data from this study. All other data from Brenner *et al.* (1999).

Emended description of *Leptospiraceae* Hovind-Hougen 1979

Comprises three genera, *Leptospira*, *Leptonema* and *Turneriella*, defined by differences in G+C content, DNA–DNA relatedness and 16S rRNA gene sequences. The G+C contents of the genera *Leptospira*, *Leptonema* and *Turneriella* are 33–43, 54 and 48 mol%, respectively (Yasuda *et al.*, 1987). Other characteristics are as described previously (Hovind-Hougen, 1979; Johnson & Faine, 1984). The type genus is *Leptospira* Noguchi 1917.

Description of *Turneriella* gen. nov.

Turneriella (Tur'ne.ri.el.la. N.L. fem. n. *Turneriella* to honour Leslie Turner, an English medical microbiologist who made definitive contributions to the knowledge of leptospirosis).

Cells are Gram-negative, flexible and helical, measuring 0.3×3.5 – 7.5 μm ; wavelength is 0.3 – 0.5 μm (Hovind-Hougen *et al.*, 1981). Motile by means of two axial filaments. Obligately aerobic. Oxidase-positive. The type species is *Turneriella parva* (Hovind-Hougen *et al.* 1982) Levett *et al.* 2005.

Description of *Turneriella parva* comb. nov.

Turneriella parva (par'va. L. fem. adj. *parva* small).

Basonym: *Leptospira parva* Hovind-Hougen *et al.* 1982.

Exhibits the following characteristics in addition to those given in the genus description. DNA G+C content is 48 mol% (Yasuda *et al.*, 1987). Growth is inhibited by 200 μg 8-azaguanine ml^{-1} and 10 μg 2,6-diaminopurine ml^{-1} . Produces lipase. Grows at 13, 30 and 37 °C after prolonged incubation. Strains have been isolated from contaminated culture medium, from tap water and from the uterus of a sow. Not pathogenic for hamsters. The type strain is H^T (=NCTC 11395^T =ATCC BAA-1111^T).

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References

- Brenner, D. J., McWhorter, A. C., Knutson, J. K. & Steigerwalt, A. G. (1982). *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. *J Clin Microbiol* **15**, 1133–1140.
- Brenner, D. J., Kaufmann, A. F., Sulzer, K. R., Steigerwalt, A. G., Rogers, F. C. & Weyant, R. S. (1999). Further determination of DNA relatedness between serogroups and serovars in the family *Leptospiraceae* with a proposal for *Leptospira alexanderi* sp. nov. and four new *Leptospira* genomospecies. *Int J Syst Bacteriol* **49**, 839–858.
- Hookey, J. V. (1993). Characterization of *Leptospiraceae* by 16S DNA restriction length polymorphisms. *J Gen Microbiol* **139**, 1681–1689.
- Hookey, J. V., Bryden, J. & Gatehouse, L. (1993). The use of 16S rDNA sequence analysis to investigate the phylogeny of *Leptospiraceae* and related spirochaetes. *J Gen Microbiol* **139**, 2585–2590.
- Hovind-Hougen, K. (1979). *Leptospiraceae*, a new family to include *Leptospira* Noguchi 1917 and *Leptonema* gen. nov. *Int J Syst Bacteriol* **29**, 245–251.
- Hovind-Hougen, K., Ellis, W. A. & Birch-Andersen, A. (1981). *Leptospira parva* sp. nov.: some morphological and biological characters. *Zentralbl Bakteriol Mikrobiol Hyg A* **250**, 343–354.
- Johnson, R. C. & Faine, S. (1984). Genus I. *Leptospira* Noguchi 1917, 755.^{AL} In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 62–67. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Marshall, R. (1992). International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of *Leptospira*. Minutes of the meetings, 13 and 15 September 1990, Osaka, Japan. *Int J Syst Bacteriol* **42**, 330–334.
- Perolat, P., Chappel, R. J., Adler, B., Baranton, G., Bulach, D. M., Billinghamurst, M. L., Letocart, M., Merien, F. & Serrano, M. S. (1998). *Leptospira fainei* sp. nov., isolated from pigs in Australia. *Int J Syst Bacteriol* **48**, 851–858.
- Ramadass, P., Jarvis, B. D. W., Corner, R. J., Penny, D. & Marshall, R. B. (1992). Genetic characterization of pathogenic *Leptospira* species by DNA hybridization. *Int J Syst Bacteriol* **42**, 215–219.
- Yasuda, P. H., Steigerwalt, A. G., Sulzer, K. R., Kaufmann, A. F., Rogers, F. & Brenner, D. J. (1987). Deoxyribonucleic acid relatedness between serogroups and serovars in the family *Leptospiraceae* with proposals for seven new *Leptospira* species. *Int J Syst Bacteriol* **37**, 407–415.