Systematics of *Leptospiraceae*

Paul N. Levett

**Abstract** Leptospires are spirochetes that may be free-living saprophytes found in freshwater or may cause acute or chronic infection of animals. The family *Leptospiraceae* comprises three genera: *Leptospira*, *Leptonema*, and *Turneriella*. Within the genus *Leptospira*, three clades can be distinguished, of pathogens, nonpathogens, and an intermediate group. Leptospires are further divided into serovars; antigenically related serovars are clustered into serogroups for convenience.

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1 Systematics of *Leptospiraceae*

1.1 Taxonomy

Leptospires are spirochaetes that may be free-living saprophytes found in freshwater or may cause acute or chronic infections of animals (Zuerner 2010). The family *Leptospiraceae* was defined in 1979 to include the genera *Leptospira* and...
Leptonema (Hovind-Hougen 1979), and was included in the Approved Lists of Bacterial Names (Skerman et al. 1980). It now contains three genera of spirochaetes: Leptospira, Leptonema, and Turneriella (Levett et al. 2005). The type genus is Leptospira Noguchi (1917).

The three genera are 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 53.6 mol%, respectively (Stackebrandt et al. 2013; Yasuda et al. 1987). Other characteristics are described in detail elsewhere (Hovind-Hougen 1979; Johnson and Faine 1984; Zuerner 2010).

1.2 Nomenclature

The species of Leptospira are divided into a large number of serovars, defined by agglutination after cross-absorption of rabbit antisera with heterologous antigen (Dikken and Kmety 1978; Kmety and Dikken 1993). If more than 10% of the homologous titre remains in at least one of the two antisera on repeated testing, two strains are said to belong to different serovars (Wolff and Broom 1954). 193 serovars were cataloged within the species Leptospira interrogans sensu lato (Kmety and Dikken 1993), and over 60 serovars of Leptospira biflexa sensu lato were recorded (Faine and Stallman 1982). Many additional serovars have been isolated and characterized, and after confirmation in at least one international reference laboratory, are recognized by the Taxonomic Subcommittee.

Serovar names should be written with an initial capital letter and should not be italicized. It is incorrect to write the serovar name after Leptospira (Levett and Smythe 2006). An example of the correct nomenclature for a serovar is L. interrogans serovar Icterohaemorrhagiae.

Serovars that are antigenically related have traditionally been grouped into serogroups (Kmety and Dikken 1993; Wolff and Broom 1954) for convenience. Serogroups have no taxonomic standing, but they have proved useful for initial serological diagnosis and for epidemiological understanding at the regional or population level.

2 Classification of Leptospiraceae

2.1 Historical Classification

The isolation of leptospires was first reported from freshwater in 1914 by Wolbach and Binger (1914), who named the organism Spirocheta biflexa. Similar organisms were isolated from the blood of miners suffering from Weil’s disease in Japan by
Inada and Ido (1915). The announcement of this discovery was made in January 1915 at the Kyushu University Medical School in Fukuoka, Japan, and the first publication appeared in February 1915 (Kobayashi 2001). Inada and Ido initially named this organism *Spirochaeta icterohaemorrhagica japonica*, but this was changed to *Spirochaeta icterohaemorrhagiae* before the first publication in English (Inada et al. 1916). This finding was rapidly confirmed by studies of soldiers fighting in the trenches in Western Europe (Hübener and Reiter 1915; Uhlenhuth and Fromme 1915). The genus name *Leptospira* was proposed by Noguchi (1917) after study of isolates from Japan, Europe and the USA. See the chapter by B. Adler, this volume for a more detailed description of the history of the discovery of pathogenic *Leptospira*.

In subsequent years, isolates from many different locations were named. Differentiation between strains was by achieved by a variety of agglutination tests and antigenically distinct strains were assigned the status of species. By 1948, four species, *L. icterohaemorrhagiae*, *Leptospira hebdomadis*, *L. biflexa*, and *Leptospira canicola* were recognized in Bergey’s Manual (Robinson 1948). A further 23 species with inadequate descriptions were listed, with the caution that many were probably synonyms of the four recognized species. Concern about the proliferation of new “species” and the relative difficulty in differentiating the newly described strains from existing strains, led Wolff and Broom (1954) to propose a standardized approach to maintenance of cultures and serological characterization based upon the methods established in Wolff’s laboratory in Amsterdam. Wolff and Broom considered that assigning species names for serologically distinct strains was unjustified and suggested the use of the term serotype for the basic taxonomic unit of a serological classification. Using the Amsterdam system, “two strains are considered to belong to different serotypes if, after cross-absorption with adequate amounts of heterologous antigen, 10 % or more of the homologous titre regularly remains in each of the two antisera” (Wolff and Broom 1954); as a result, 32 distinct serotypes were recognized. Wolff and Broom further suggested that closely related serotypes could be clustered into serogroups for convenience.

The definition of a serotype was the subject of discussion at several meetings of the Taxonomic Subcommittee on *Leptospira*. At the 1966 meeting, the definition was modified to state “Two strains are considered to belong to different serotypes if, after cross-absorption with adequate amounts of heterologous antigen, 10 % or more of the homologous titer regularly remains in at least one of the two antisera in repeated tests” (Turner 1971). A further modification was made at the 1986 meeting: “Two strains are said to belong to different serovars if after cross-absorption with adequate amounts of heterologous antigen more than 10 % of the homologous titer regularly remains in at least one of the two antisera in repeated tests” (Stallman 1987). These small changes to the definition of a serovar have unfortunate consequences, in that several serovars defined using the historical definition would no longer be considered distinct from each other using the more recent definition (Hartskeerl et al. 2004). This inconsistency may well be resolved by whole genome sequencing.
In the 7th edition of Bergey’s Manual (Wolff and Broom 1957), the genus *Leptospira* was divided into two species, *L. icterohaemorrhagiae*, comprising all pathogenic strains, and *L. biflexa*, containing saprophytic strains isolated from water. *L. icterohaemorrhagiae* was further subdivided into serotypes, but *L. biflexa* was not. At a meeting in 1962, the Taxonomic Subcommittee on *Leptospira* recommended that the pathogenic strains should be named *L. interrogans* and the saprophytic strains *L. biflexa* (Wolff and Turner 1963). At this time, the species were differentiated by growth in the presence of divalent copper ions. In the 1984 edition of Bergey’s Manual, the species were differentiated by several phenotypic characteristics: *L. biflexa* was capable of growth at 13 °C and of growth in the presence of 8-azaguanine (225 μg/ml) and failed to form spherical cells in 1 M NaCl (Johnson and Faine 1984).

### 2.2 Genetic Basis for Classification

The phenotypic classification of leptospires has been replaced by a genotypic one, in which a number of so-called genomospecies includes all serovars of both *L. interrogans* sensu lato and *L. biflexa* sensu lato. Genetic heterogeneity among leptospiral serovars was demonstrated over 40 years ago, when pathogenic and nonpathogenic “complexes” were shown to have little DNA homology (Haapala et al. 1969). Based on base pair ratios, there were at least two groups within the pathogenic strains. Further work expanded the number of homology groups to six (Brendle et al. 1974). In addition, serovar Illini was shown to be genetically distinct from other leptospires (Brendle et al. 1974), leading to the definition of the monospecific genus *Leptonema* (Hovind-Hougen 1979). A further strain was found to be serologically and genetically distinct both from other leptospires and from *Leptonema illini*, and was named *Leptospira parva* (Hovind-Hougen et al. 1981). This species was later transferred to a new genus, *Turneriella* (Levett et al. 2005). Subsequent DNA–DNA hybridization studies led to the definition of 10 species of *Leptospira* (Yasuda et al. 1987). Heterogeneity within the species *L. biflexa* was confirmed independently (Ramadass et al. 1990). An additional species, *L. kirschneri*, was added soon after (Ramadass et al. 1992). After an extensive study of several hundred strains, five new species were described (Brenner et al. 1999), one of which was named *L. alexanderi*. Unfortunately, Brenner et al. did not assign names to four of the new genomospecies that were each represented by only one or two isolates. This led to confusion in the literature, which has been resolved only recently (Smythe et al. 2013).

A number of other species have been described: *Leptospira fainei* (Pérolat et al. 1998), *Leptospira broomii* (Levett et al. 2006), *Leptospira wolfii* (Slack et al. 2008), *Leptospira licerasiae* (Matthias et al. 2008), *Leptospira kmetyi* (Slack et al. 2009b), and *Leptospira idonii* (Saito et al. 2013). There are currently 21 species of *Leptospira* (Table 1).
The species of *Leptospira* cluster into three groups, comprising pathogens, non-pathogens and an intermediate group (Fig. 1). Similar phylogenies can be produced using several housekeeping genes, including *rrs* (Morey et al. 2006), *rpoB* (La Scola et al. 2006), and *gyrB* (Slack et al. 2006).

The species of *Leptospira* currently recognized do not correspond to the previous two species (*L. interrogans* sensu lato and *L. biflexa* sensu lato). Interestingly, both pathogenic and nonpathogenic serovars occur within several species (Table 2). However, it is also clear that some reference strains have been mislabeled, leading to erroneous classification (Slack et al. 2009a). It is likely that some of the serovars listed in Table 2 will in the future be re-classified into a single species. Genetic heterogeneity within serovars has been demonstrated (Brenner et al. 1999; Bulach et al. 2000; Feresu et al. 1999). The presence of the same LPS biosynthetic genes in strains of different species implies genetic transfer; evidence of inter-species transfer has been detected (Haake et al. 2004). Thus, neither serogroup nor serovar

### Table 1 Species within the family *Leptospiraceae*

<table>
<thead>
<tr>
<th>Species</th>
<th>Valid publication</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. alexanderi</em></td>
<td>Brenner et al. (1999)</td>
</tr>
<tr>
<td><em>L. alstonii</em></td>
<td>Smythe et al. (2013)</td>
</tr>
<tr>
<td><em>L. biflexa</em></td>
<td>Faine and Stallman (1982)</td>
</tr>
<tr>
<td><em>L. borgpetersenii</em></td>
<td>Yasuda et al. (1987)</td>
</tr>
<tr>
<td><em>L. broomii</em></td>
<td>Levett et al. (2006)</td>
</tr>
<tr>
<td><em>L. fainei</em></td>
<td>Pérolat et al. (1998)</td>
</tr>
<tr>
<td><em>L. idonii</em></td>
<td>Saito et al. (2013)</td>
</tr>
<tr>
<td><em>L. inadai</em></td>
<td>Yasuda et al. (1987)</td>
</tr>
<tr>
<td><em>L. interrogans</em></td>
<td>Faine and Stallman (1982)</td>
</tr>
<tr>
<td><em>L. kirschneri</em></td>
<td>Ramadass et al. (1992)</td>
</tr>
<tr>
<td><em>L. knetji</em></td>
<td>Slack et al. (2009b)</td>
</tr>
<tr>
<td><em>L. licerasiae</em></td>
<td>Matthias et al. (2008)</td>
</tr>
<tr>
<td><em>L. meyeri</em></td>
<td>Yasuda et al. (1987)</td>
</tr>
<tr>
<td><em>L. noguchii</em></td>
<td>Yasuda et al. (1987)</td>
</tr>
<tr>
<td><em>L. santarosai</em></td>
<td>Yasuda et al. (1987)</td>
</tr>
<tr>
<td><em>L. terpstraee</em></td>
<td>Smythe et al. (2013)</td>
</tr>
<tr>
<td><em>L. vanthiellii</em></td>
<td>Smythe et al. (2013)</td>
</tr>
<tr>
<td><em>L. weilii</em></td>
<td>Yasuda et al. (1987)</td>
</tr>
<tr>
<td><em>L. wolvachii</em></td>
<td>Yasuda et al. (1987)</td>
</tr>
<tr>
<td><em>L. wofflii</em></td>
<td>Slack et al. (2008)</td>
</tr>
<tr>
<td><em>L. yanagawae</em></td>
<td>Smythe et al. (2013)</td>
</tr>
<tr>
<td><em>Turneriella parva</em></td>
<td>Levett et al. (2005)</td>
</tr>
<tr>
<td><em>Leptonema illini</em></td>
<td>Hovind-Hougen (1979)</td>
</tr>
</tbody>
</table>

### 2.3 Phylogenetic Classification

The species of *Leptospira* cluster into three groups, comprising pathogens, non-pathogens and an intermediate group (Fig. 1). Similar phylogenies can be produced using several housekeeping genes, including *rrs* (Morey et al. 2006), *rpoB* (La Scola et al. 2006), and *gyrB* (Slack et al. 2006).

The species of *Leptospira* currently recognized do not correspond to the previous two species (*L. interrogans* sensu lato and *L. biflexa* sensu lato). Interestingly, both pathogenic and nonpathogenic serovars occur within several species (Table 2). However, it is also clear that some reference strains have been mislabeled, leading to erroneous classification (Slack et al. 2009a). It is likely that some of the serovars listed in Table 2 will in the future be re-classified into a single species. Genetic heterogeneity within serovars has been demonstrated (Brenner et al. 1999; Bulach et al. 2000; Feresu et al. 1999). The presence of the same LPS biosynthetic genes in strains of different species implies genetic transfer; evidence of inter-species transfer has been detected (Haake et al. 2004). Thus, neither serogroup nor serovar
of an isolate currently predicts the species of *Leptospira*. In addition, the phenotypic characteristics formerly used to differentiate *L. interrogans* sensu lato from *L. biflexa* sensu lato do not differentiate the genomospecies (Brenner et al. 1999; Yasuda et al. 1987).

Characterization of leptospiral isolates requires both identification of species and serovar. In addition to sequence-based identification, a wide range of molecular approaches has been applied to species identification (Ahmed et al. 2012). The first leptospiral genome was sequenced over 10 years ago (Xue et al. 2009). The sequencing of 200 further strains has recently been completed through the *Leptospira* Genomics and Human Health Project (http://gsc.jcvi.org/projects/gsc/leptospira/) and the genomes of *L. illini* and *Turneriella parva* have also been

![Molecular phylogenetic analysis of Leptospiraceae 16S rRNA gene sequences by maximum likelihood method, based on the Tamura-Nei model, using MEGA5 (Tamura et al. 2011). The tree with the highest log likelihood is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. There was a total of 1,230 positions in the final dataset.](image-url)
sequenced recently (Huntemann et al. 2013; Stackebrandt et al. 2013). The analysis of these sequences will further understanding of the taxonomy of the Leptospiraceae and will provide molecular tools for species identification and for serovar characterization.

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bataviae</td>
<td>L. interrogans, L. santarosai</td>
</tr>
<tr>
<td>Bulgrica</td>
<td>L. interrogans, L. kirschner</td>
</tr>
<tr>
<td>Grippotyphosa</td>
<td>L. interrogans, L. kirschner</td>
</tr>
<tr>
<td>Hardjo</td>
<td>L. borgpetersenii, L. interrogans, L. meyer</td>
</tr>
<tr>
<td>Icterohaemorrhagiae</td>
<td>L. interrogans, L. inadai</td>
</tr>
<tr>
<td>Kremastos</td>
<td>L. interrogans, L. santarosai</td>
</tr>
<tr>
<td>Mwogolo</td>
<td>L. interrogans, L. kirschner</td>
</tr>
<tr>
<td>Paidjan</td>
<td>L. interrogans, L. kirschner</td>
</tr>
<tr>
<td>Pomona</td>
<td>L. interrogans, L. noguchii</td>
</tr>
<tr>
<td>Pyrogenes</td>
<td>L. interrogans, L. santarosai</td>
</tr>
<tr>
<td>Szwajizak</td>
<td>L. interrogans, L. santarosai</td>
</tr>
<tr>
<td>Valbuzzi</td>
<td>L. interrogans, L. kirschner</td>
</tr>
</tbody>
</table>

It is probable that some of the serovars listed above were actually mislabeled in reference collections. Future examination of reference strains may result in revisions to this list

References


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