Mycoplasma leachii sp. nov. as a new species designation for Mycoplasma sp. bovine group 7 of Leach, and reclassification of Mycoplasma mycoides subsp. mycoides LC as a serovar of Mycoplasma mycoides subsp. capri

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The Mycoplasma mycoides cluster consists of six pathogenic mycoplasmas causing disease in ruminants, which share many genotypic and phenotypic traits. The M. mycoides cluster comprises five recognized taxa: Mycoplasma mycoides subsp. mycoides Small Colony (MmmSC), M. mycoides subsp. mycoides Large Colony (MmmLC), M. mycoides subsp. capri (Mmc), Mycoplasma capricolum subsp. capricolum (Mcc) and M. capricolum subsp. capripneumoniae (Mccp). The group of strains known as Mycoplasma sp. bovine group 7 of Leach (MBG7) has remained unassigned, due to conflicting data obtained by different classification methods. In the present paper, all available data, including recent phylogenetic analyses, have been reviewed, resulting in a proposal for an emended taxonomy of this cluster: (i) the MBG7 strains, although related phylogenetically to M. capricolum, hold sufficient characteristic traits to be assigned as a separate species, i.e. Mycoplasma leachii sp. nov. (type strain, PG50T = N29T = NCTC 10133T = DSM 21131T); (ii) MmmLC and Mmc, which can only be distinguished by serological methods and are related more distantly to MmmSC, should be combined into a single subspecies, i.e. Mycoplasma mycoides subsp. capri, leaving M. mycoides subsp. mycoides (MmmSC) as the exclusive designation for the agent of contagious bovine pleuropneumonia. A taxonomic description of M. leachii sp. nov. and emended descriptions of M. mycoides subsp. mycoides and M. mycoides subsp. capri are presented. As a result of these emendments, the M. mycoides cluster will hereafter be composed of five taxa comprising three subclusters, which correspond to the M. mycoides subspecies, the M. capricolum subspecies and the novel species M. leachii.

At present, the Mycoplasma mycoides cluster comprises six closely related mycoplasmas that are currently referred to as M. mycoides subsp. mycoides Small Colony (MmmSC) and Large Colony (MmmLC), M. mycoides subsp. capri (Mmc), Mycoplasma capricolum subsp. capricolum (Mcc), M. capricolum subsp. capripneumoniae (Mccp) and Mycoplasma sp. bovine group 7 of Leach (MBG7), the latter being a group of strains that have remained
unassigned. All of them are ruminant pathogens. Of greatest importance are MmmSC and Mccp, the aetiolo-
gical agents of contagious bovine and contagious caprine
pleuropneumonia (CBPP and CCPP), respectively, which are
listed by the Office International des Epizooties (OIE)
as notifiable animal diseases. The other members of this
cluster, i.e. MmmLC, Mmc and Mcc, cause various
symptoms in small ruminants, including mastitis, arthritis,
keratoconjunctivitis, pneumonia and septicaemia
(Thiaucourt & Bolske, 1996). Strains of MBG7 that cause
mastitis and polyarthritis in cattle are serologically distinct
from other bovine mycoplasmas (Leach, 1967).

The members of the M. mycoides cluster share many
genomic and phenotypic traits, thus creating taxonomic
and diagnostic problems. The subspecies Mccp was
designated only recently (Leach et al., 1993), whereas
the assignment of MBG7 strains and the position of
MmmLC and Mmc as separate subspecies of M. mycoides
have been debated for years. The consensus of the
Subcommittee on the Taxonomy of Mollicutes of the
International Committee on Systematics of Prokaryotes,
as stated at its meeting in Cambridge, UK, in 2006, was
that a proposal for an emended taxonomy of this cluster
should be prepared in conjunction with a peer-reviewed
publication (Brown & Bradbury, 2007). Therefore, the
aim of this article is to establish MBG7 as a novel species,
designated Mycoplasma leachii sp. nov., and to combine
MmmLC and Mmc into a single taxon, namely M. mycoides subsp. capri.

Species differentiation in the class Mollicutes has classically
been accomplished by using serological assays such as the
growth-inhibition test. Nevertheless, within the M. mycoides cluster, these assays are frequently hampered by interspecies cross-reactions as well as intraspecies variabil-
ity (Cottew et al., 1987). DNA–DNA hybridization (DDH),
introduced in the late 1960s, was used to define relations-
ships among members of this cluster and supported the
definition of subspecies within M. mycoides and M. capricolum. However, the technique is challenging and
allows only global comparison between genomic DNA
samples, so that the data may not always be conclusive
(Cottew et al., 1987). When 16S rRNA gene (rrs) sequence-
based classification was introduced into bacterial system-
atics, it constituted an additional important basis for the
definition of relationships among mollicute species. How-
ever, the resolution of 16S rRNA gene sequences within the M. mycoides cluster proved insufficient for phylogeny (Pettersson et al., 1996). Therefore, phylogenetic
analyses based on 16S–23S rRNA intergenic spacer regions,
protein-encoding genes and intergenic sequences, which
are not as highly conserved, were conducted to resolve the
 evolutionary relationships among the members of this
cluster (Harasawa et al., 2000; Manso-Silván et al., 2007;
Thiaucourt et al., 2000; Vilei et al., 2006). Omitting any
reference to changes in nomenclature (note that abbrevi-
ated names correspond to current designations) and in
the understanding of the diseases associated with these
organisms, the evolution of the taxonomy of the M. mycoides cluster may be considered as follows.

MmmSC, the aetiological agent of CBPP, was the first
mycoplasma to be isolated (Nocard et al., 1898) and its
type strain, PG1T, also represents the type for the class
Mollicutes. The Large Colony type, represented by strain Y-
goatR (Laws, 1956), was included in the same subspecies
due to serological cross-reactions with PG1T, despite
differences in pathogenicity, host predilection and other
characteristics (Cottew & Yeats, 1978). In contrast, Mmc
strain PG3T had been classified as a separate subspecies on
the basis of antigenic constitution and host specificity
(Edward, 1954; Freundt, 1955). Although supported by
DDH studies (Askaa et al., 1978), the distinction of
MmmLC and Mmc as separate subspecies has been debated
for years, as numerous investigations, based on both
genetic and phenotypic analyses, suggested that MmmLC
was related more closely to Mmc than to MmmSC (Abu-
Groun et al., 1994; Leach et al., 1989; Manso-Silván et al.,
2007; Olsson et al., 1990; Pettersson et al., 1996; Rodwell,
1982; Vilei et al., 2006). Mcc, represented by type strain
California kidT, was recognized early to be a separate
species within the M. mycoides cluster, grouping caprine
mycoplasmas with characteristic biological, biochemical and
serological properties (Tully et al., 1974). On the other
hand, Mccp, the aetiological agent of CCPP, was known for
many years as the ‘F38-type mycoplasmas’ in reference to
strain F38T. This taxon remained unassigned until DDH
studies conducted on field and type strains supported a
subspecies relationship of this agent with Mcc (Bonnet
et al., 1993), which had already been suggested based on
genomic and phenotypic data (Christiansen & Erno, 1982,
1990; Costas et al., 1987; Taylor et al., 1992). MBG7, which
is represented by strain PG50, remained unassigned due to
inconsistent genotypic and phenotypic data (Cottew et al.,
1987) and serological cross-reactions between Mccp and
the MBG7 strains (Erno et al., 1983; Kibe et al., 1985), as
well as the fact that MBG7 shared antigens with MmmSC
(Cheng et al., 1996; Djordjevic et al., 2003; Frey et al., 1998;
Vilei et al., 2000). Phylogenetic studies suggested that
MBG7 and M. capricolum are related more closely to each
other than to other members of the M. mycoides cluster
(Harasawa et al., 2000; Manso-Silván et al., 2007;
Pettersson et al., 1996; Thiaucourt et al., 2000).

Recent phylogenetic data from multiple strains of each
taxon, together with previous phenotypic and genotypic
information, provide support for a case to emend the
taxonomy of the M. mycoides cluster, namely to establish a
new species designation for MBG7 and to combine
MmmLC and Mmc into a single entity within M. mycoides.

Mycoplasma leachii sp. nov., a new species name
for MBG7

A group of mycoplasma strains, including the reference
strain PG50, were isolated from cattle suffering from
arthritis (Simmons & Johnston, 1963) and mastitis
(Connole et al., 1967) in Australia. As these strains were shown to be serologically distinct from other bovine mycoplasmas (Leach, 1967), they have been known as ‘serogroup 7 of Leach’ (MBG7). Even though MBG7 is still associated with severe outbreaks of polyarthritis, mastitis and abortion in dairy cattle in Australia (Hum et al., 2000), this group of strains has remained unassigned. DDH analysis showed equal distances (approx. 60%) between MBG7 and M. mycoides and M. capricolum, suggesting a separate species designation (Christiansen & Erno, 1982). Electrophoretic analysis of isoenzymes (Salih et al., 1983), SDS-PAGE profile comparisons (Costas et al., 1987) and distinctive substrate-oxidation patterns (Abu-Groun et al., 1994) also supported its classification as a separate species. However, conflicting data from different studies revealed a close relatedness either to M. mycoides or to M. capricolum.

First, DDH analyses showed 85% hybridization between MBG7 strain PG50 and MmmSC strain PG15 (Askaa et al., 1978), although the results presented in this study fluctuated and the DNA hybridization was considered to be too high, compared with two-dimensional PAGE protein data (Rodwell, 1982). LppA sequence similarity and serological cross-reactions (Fréy et al., 1998), as well as sequence identity of the glycerol-transport locus gtsABC (Djordjevic et al., 2003) and of rpoB (Vilei et al., 2006), also indicated a close relatedness to MmmSC. This might be due to horizontal gene transfer, which has been described among distant mycoplasma species sharing the same host (Sirand-Pugnet et al., 2007; Thomas et al., 2005).

On the other hand, serological cross-reactions had previously suggested that MBG7 was closer to the M. capricolum subspecies (Christiansen & Erno, 1982), which was confirmed by one- and two-dimensional SDS-PAGE profile comparisons (Costas et al., 1987; Olsson et al., 1990). In addition, analysis of 16S rRNA genes revealed a strong sequence similarity to M. capricolum, with only four variable positions found between reference strain PG50 and Mcc strain California kid (Pettersson et al., 1996). Moreover, recent phylogenetic studies based on 16S–23S rRNA intergenic spacer regions of M. mycoides cluster failed to distinguish these two taxa (Abu-Groun et al., 1994) and an analysis of 16S rRNA gene sequences indicated that Mmc and MmmLC should be merged into the same subspecies (Pettersson et al., 1996). Phylogenetic studies of the M. mycoides cluster based on alternative loci also showed that MmmLC and Mmc strains could not be differentiated. Comparison of 16S–23S rRNA intergenic spacer regions of the different type strains (Harasawa et al., 2000), as well as phylogenetic analysis of protein-encoding genes conducted on multiple strains for each group (Manso-Silván et al., 2007; Thiaucourt et al., 2000; Vilei et al., 2006), concurred in favour of a reclassification of these two taxa into a single subspecies of M. mycoides. The name Mycoplasma mycoides subsp. capri, which has already been proposed, may be the most appropriate, as it allows retention of most of the current designations whilst avoiding confusion between MmmLC, which is associated with several diseases in small ruminants, and MmmSC, a most important bovine pathogen and causative agent of CBPP.

In conclusion, MBG7 merits assignment as a separate species within the M. mycoides cluster, namely Mycoplasma leachii sp. nov., because it is placed at an intermediary position between M. mycoides and M. capricolum as assessed by DDH and protein patterns, exhibits differences in metabolic pathways and antigenicity and is associated with severe disease in cattle.

Mycoplasma mycoides subsp. capri, a collective designation for the former taxa of M. mycoides subsp. mycoides LC and M. mycoides subsp. capri

The distinction between MmmLC and Mmc has always been tenuous. Numerous investigations have highlighted their close relatedness (Costas et al., 1987; Leach et al., 1989; Olsson et al., 1990; Rodwell, 1982; Salih et al., 1983; Taylor et al., 1992), suggesting that they may represent different serovars rather than subspecies. A broad study of the biochemical diversity within the M. mycoides cluster failed to distinguish these two taxa (Abu-Groun et al., 1994) and an analysis of 16S rRNA gene sequences indicated that Mmc and MmmLC should be merged into the same subspecies (Pettersson et al., 1996). Phylogenetic studies of the M. mycoides cluster based on alternative loci also showed that MmmLC and Mmc strains could not be differentiated. Comparison of 16S–23S rRNA intergenic spacer regions of the different type strains (Harasawa et al., 2000), as well as phylogenetic analysis of protein-encoding genes conducted on multiple strains for each group (Manso-Silván et al., 2007; Thiaucourt et al., 2000; Vilei et al., 2006), concurred in favour of a reclassification of these two taxa into a single subspecies of M. mycoides. The name Mycoplasma mycoides subsp. capri, which has already been proposed, may be the most appropriate, as it allows retention of most of the current designations whilst avoiding confusion between MmmLC, which is associated with several diseases in small ruminants, and MmmSC, a most important bovine pathogen and causative agent of CBPP.

In conclusion, MmmLC and Mmc should be grouped into the single subspecies M. mycoides subsp. capri, as both agents cause similar diseases in small ruminants and have the same morphological, cultural, biochemical and genotypic properties.

Emended taxonomy of the Mycoplasma mycoides cluster

As a result of the emendations presented here, the M. mycoides cluster will hereafter be composed of five taxa comprising three subclusters, which correspond to the M. mycoides subspecies, the M. capricolum subspecies and the novel species M. leachii. The differential traits of the members of this cluster are shown in Table 1.
Table 1. Differential traits among members of the Mycoplasma mycoides cluster

Abbreviations: h, high metabolic rate; MAKePS, mastitis, arthritis, keratitis, pneumonia and septicaemia syndrome; ND, not determined; s, slow; v, variable, depending on strain; w, weak or negative at low substrate concentrations.

<table>
<thead>
<tr>
<th>Trait</th>
<th>MmmSC</th>
<th>Mmc</th>
<th>M. leachii</th>
<th>Mcc</th>
<th>Mccp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main host</td>
<td>Bovine</td>
<td>Caprine (ovine)</td>
<td>Bovine</td>
<td>Caprine (ovine)</td>
<td>Caprine</td>
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<tr>
<td>Disease</td>
<td>CBPP</td>
<td>MAKePS</td>
<td>Mastitis, arthritis</td>
<td>MAKePS</td>
<td>CCPP</td>
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<td>Growth at:</td>
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<td>37 °C</td>
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<td>22 °C</td>
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<td>–</td>
<td>ND</td>
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<td>Filamentous growth</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<td>+</td>
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<tr>
<td>Arginine hydrolysis</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+ (s)</td>
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<td>Maltose oxidation*</td>
<td>–</td>
<td>+ (v)</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Trehalose oxidation*</td>
<td>–</td>
<td>v</td>
<td>+ (h)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mannose and glucosamine oxidation*</td>
<td>+ (w)</td>
<td>+</td>
<td>(+)</td>
<td>+ (w)</td>
<td>–</td>
</tr>
<tr>
<td>Coagulated serum and casein digestion</td>
<td>+ (w)</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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</table>

*Abu-Groun et al. (1994).

Description of Mycoplasma leachii sp. nov.

Mycoplasma leachii (lea’chi.i. N.L. masc. gen. n. leachii of Leach, named in honour of Dr R. H. Leach, who first characterized this taxon).

This description is based on data obtained from previous reports (Abu-Groun et al., 1994; Cottew et al., 1987; Leach, 1967). Non-spiral, non-motile, pleomorphic cells that lack a cell wall, are bounded by a single plasma membrane and pass through 0.45 nm pore-size membranes. As a facultative anaerobe, it grows rapidly, producing heavily turbid cultures and relatively large colonies with characteristic fried-egg morphology, which measure about 2 mm in diameter on suitable solid media such as Hayflick medium (Hayflick, 1965). It does not produce films or spots, nor does it form filaments. Optimal growth occurs at 37 °C; it may grow at 28 °C, but not at 22 °C. Susceptible to digitonin, requires sterols for growth, digests casein, liquefies inspissated serum and produces haemolysis on horse blood agar. Ferments glucose, but does not metabolize arginine or urea. Can be distinguished from other members of the M. mycoides cluster by a high rate of trehalose metabolism, coupled to an inability to oxidize mannose and glucosamine at concentrations <50 μM. Members of this species produce strong serological cross-reactivity with M. capricolum subsp. capripneumoniae and limited reactivity with strains of M. capricolum subsp. capri, and share antigens with M. mycoides. The DNA G+C content was estimated to be 25.4 mol% (Askaa et al., 1978; Christiansen & Erno, 1982). Associated mainly with mastitis, polyarthritis and abortion in dairy cattle, although it may also be found in healthy cattle, as well as in small ruminant hosts.

The type strain PG50T, previously known as N29T (=NCTC 10133T =DSM 21131T), was isolated in Australia in 1963 from an arthritic joint of a calf.

Emended description of Mycoplasma mycoides subsp. mycoides (Borrel et al. 1910) Freundt 1955 (Approved Lists 1980)

Initially designated Asterococcus mycoides (Borrel et al., 1910), referring to its pleomorphic, mucous nature and to the production of mycelium-like filaments, the nomenclature of this taxon was modified to Mycoplasma mycoides when it acquired the legitimate genus name Mycoplasma (Edward & Freundt, 1956), as listed on the Approved Lists of Bacterial Names that became effective in January 1980 (Skerman et al., 1980). This description, based on the original descriptions and on additional data, emphasizes the particularities related to the fact that this taxon no longer includes the Large Colony strains represented by strain Y-goatR.

A non-helical, pleomorphic organism measuring 125–175 nm in length (Edward, 1954). Facultative anaerobe that displays a tendency to produce filamentous growth, but does not produce films or spots. Optimal growth occurs at 37 °C; no growth is evident at 22 °C. Its viability is extremely reduced after 12 h incubation at 45 °C (Cottew & Yeats, 1978). Grows relatively slowly and, after 2 days incubation, produces small colonies about 0.5 mm in diameter (Thigpen et al., 1983), which show the characteristic fried-egg appearance on suitable solid medium such as that of Hayflick (1965). When broth cultures are shaken, the sediment rises in characteristic ‘silky swirls’. Digests casein and liquefies inspissated serum, albeit less vigorously than the other members of the M. mycoides cluster. Produces haemolysis on horse blood agar and filtrates of 6 day cultures discoulour horse erythrocytes. Ferments glucose, does not metabolize arginine or urea and can be distinguished from other members of the M. mycoides cluster by its total inability to oxidize maltose and trehalose, coupled to its inability to oxidize mannose and glucosamine at concentrations <50 μM (Abu-Groun et al., 1994). Shows serological cross-reactions with M. mycoides subsp. capri (Cottew &
Yeats, 1978; Thigpen et al., 1983) and shares antigens with the newly designated taxon *M. leachii* (formerly MBG7). According to *Bergey’s Manual of Determinative Bacteriology* (Holt, 1994), the DNA G+C content is 26.1–27.1 mol%; the genome sequence from type strain PG1T showed a G+C content of 24.0 mol% (Westberg et al., 2004). *Mycoplasma mycoides* subsp. *mycoides* is the aetiological agent of contagious bovine pleuropneumonia (CBPP).

The type strain, PG1T (= NCTC 10114T = CCUG 32753T), was deposited in 1931 by P. Laidlaw as a representative of the CBPP agent. The exact origin of the strain is not known and the cultures are no longer pathogenic.


Initially designated *Asterococcus mycoides* ‘variety’ *capri* (Edward, 1953), this taxon acquired the genus name *Mycoplasma* in 1956 (Edward & Freundt, 1956), as listed on the Approved Lists of Bacterial Names that became effective in January 1980 (Skerman et al., 1980). This subspecies now also includes strains previously referred to as *M. mycoides* subsp. *mycoides* Large Colony, which are represented by strain Y-goatR. Non-helical, pleomorphic, facultative anaerobe. It grows optimally at 37 °C, less vigorously at 22 °C and its viability is maintained after 2 days incubation at 45 °C (Thigpen et al., 1983). It grows rapidly in liquid medium such as that of Hayflick (1965), producing heavily turbid cultures. On suitable solid medium, colonies with characteristic fried-egg morphology about 2.5 mm in diameter appear after 2 days (Thigpen et al., 1983). Does not produce filamentous growth, films or spots. Digests casein, liquefies inspissated serum, produces haemolysis on horse blood agar and the supernatants from 6 day cultures discolour horse erythrocytes. Ferments glucose, but does not metabolize arginine or urea. Serological cross-reactions have been reported with *M. mycoides* subsp. *mycoides* (Cottew & Yeats, 1978; Thigpen et al., 1983). According to *Bergey’s Manual of Determinative Bacteriology* (Holt, 1994), the DNA G+C content is 24.0–26.0 mol%; the partial genome sequence of strain GM12 shows a G+C content of 23.0 mol% (Lartigue et al., 2007). Associated with various symptoms in small ruminants, including mastitis, arthritis, keratoconjunctivitis, pneumonia and septicaemia.

The type strain, PG1T (= NCTC 10137T = CIP 71.25T), was isolated in 1950 or possibly a case from a earlier of pleuropneumonia in a Turkish goat (Tully et al., 1974), but is no longer virulent for its primary host (El Nasri, 1967). Strain Y-goatR represents a serovar showing serological cross-reactions with *M. mycoides* subsp. *mycoides*.

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