Spiroplasma infection among white satin moth [Leucoma (= Stilpnotia) salicis L.] larvae was first discovered in 1986, which was the first record of spiroplasma infection in the insect order Lepidoptera (Lipa et al., 1988). Previous spiroplasma infections have been recorded in members of the insect orders Hymenoptera, Hemiptera, Coleoptera and Diptera and in ticks of the family Ixodidae (Hackett et al., 1990). Strain SMA\textsuperscript{T} was isolated from a fifth instar larval satin moth collected in Poland (Lipa et al., 1988). Satin moth larvae were collected from poplar trees in 1986 and 1987, and data recorded a 43 % natural infection from the 1986 collections and a range of 8–30 % infection in the 1987 collection (Lipa et al., 1988). Among laboratory-reared satin moth larvae, the infection level was higher, 55 % among 41 examined larvae, indicating that horizontal transmission of the spiroplasmas was a means to spread the infection among members of a satin moth larvae population. As this is the first natural spiroplasma infection recorded in a lepidopteran, characterization of strain SMA\textsuperscript{T} was performed.

The classification of spiroplasmas requires several morphological, biochemical and genetic tests as prescribed by the International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of Mollicutes (1995). In this paper, we report the results of taxonomic studies of strain SMA\textsuperscript{T} that satisfy the requirements for species descriptions for the class Mollicutes (International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of Mollicutes, 1995). Results support the designation of strain SMA\textsuperscript{T} as the type strain of a novel species, Spiroplasma leukoma\textsuperscript{e} sp. nov. (class Mollicutes: order Entomoplasmatales: family Spiroplasmataceae).

**Isolation and cultivation**

Techniques for isolating spiroplasmas from insect guts and haemocoel have been described previously (Hackett & Clark, 1989; Rose et al., 1993). Strain SMA\textsuperscript{T} was isolated and cultivated from the haemolymph of a larval satin moth (L. salicis) collected in Poland (Lipa et al., 1988). Primary isolation of strain SMA\textsuperscript{T} was performed in a culture of M1D medium (Whitcomb, 1983) and a co-culture of M1D medium with insect tissue cells (1 : 1) (Konai et al., 1996b). Isolates of strain SMA\textsuperscript{T} were grown at an optimal temperature of 30 °C (Konai et al., 1996a) both aerobically and anaerobically in a BBL anaerobic GasPak system (Becton Dickinson). Growth of strain SMA\textsuperscript{T} was determined by observing indicator changes in the medium (M1D) and by examining the cultures microscopically during a weekly observatory period. Initial inoculations of strain SMA\textsuperscript{T} into M1D and...
M1D/TC media involved weekly passes due to the slow growth of the organism. Other media were used for comparative growth purposes: DCCM, H-2, SFM supplemented with 10% glucose and M1D/H-2. Slower growth rates were observed in all of these media. After a 1-month adaptation in M1D medium (containing 500 U penicillin ml⁻¹), strain SMAT grew rapidly, requiring daily passes. Strain SMAT was readily filterable through 450 nm and 220 nm pore-size filters. A 100 nm pore-size membrane filtrate was free of viable cells. Strain SMAT was triply cloned in M1D medium (Whitcomb, 1983) by conventional filter-cloning procedures (Tully, 1983a) and designated strain SMAT. Representative strains of previously recognized groups and subgroups, including the type strains of previously recognized species (Williamson et al., 1998), were cultivated for comparative purposes. All spiroplasmas were grown statically in M1D medium at 30 °C.

**Morphological studies**

Dark-field microscopy (magnification ×1000) of strain SMAT observed during the exponential phase revealed helical cells with four or more turns. Strain SMAT was highly motile and formed clumps after 3 days in the same culture.

For TEM, 20 ml exponential-phase culture of strain SMAT was pelleted and fixed for 2 h in 3% glutaraldehyde, post-fixed in 1% osmium tetroxide for 3 h and then dehydrated in acetone and embedded in Spurr’s embedding medium (Williamson, 1983). Sections were stained with 2% aqueous uranyl acetate and Reynold’s lead citrate prior to visualization. As determined by TEM (Fig. 1), cells were filamentous, approximately 150 nm in diameter and surrounded by a single cytoplasmic membrane.

**Serological tests**

Strain SMAT was tested serologically against hyperimmune antisera of each of the 40 different spiroplasmas that represent established type strains and subgroup reference strains (Williamson et al., 1998) using the deformation (DF) test (Williamson et al., 1978) and the metabolism inhibition (MI) test (Williamson & Whitcomb, 1983). Positive reactions for the DF test were confirmed by the presence of grape-like clusters on the spiroplasma, indicative of a reaction between the cell membrane and the antiserum. The presence of normal, helical/spiral-shaped spiroplasmas indicated no reaction to the antiserum (Williamson et al., 1978).

The slight serological cross-reactions with group I spiroplasmas are interesting, as initial observations suggested that infection of satin moth larvae with strain SMAT increased insect mortality (J. J. Lipa, unpublished data). Additional tests are planned to determine the degree (if any) of pathogenicity of strain SMAT to several lymantriids including the gypsy moth (*Lymantria dispar*).

**Biochemical analysis**

Procedures for determining carbohydrate fermentation, arginine hydrolysis and urea utilization have been described previously (Aluotto et al., 1970). Strain SMAT was adapted to a minimal medium, SFM supplemented with 1% bovine serum fraction (Tully, 1983b) and 10% glucose. After weaning, strain SMAT was used to inoculate five tubes for biochemical testing as follows: tube 1 with SFM, tube 2 with SFM and 10% glucose, tube 3 with SFM and 10% glucose, tube 4 with SFM and 5% glucose, and tube 5 with SFM and 0% glucose.
and 21 % arginine, tube 4 with SFM and 21 % arginine and tube 5 with SFM and 1 % urea (Aluotto et al., 1970). Dark-field microscopy, as well as colour change, was used to identify growth of the cultures. A positive test showed growth in ten subsequent dilutions of the original tube and each positive test was repeated three times to ensure reproducibility. After adaptation to SFM supplemented with 10 % glucose, strain SMA was able to grow in media containing glucose, glucose and arginine, and arginine alone, indicating the abilities to ferment glucose and catabolise arginine. No growth was observed in SFM and urea, therefore urea hydrolysis was not observed.

**Genome analysis**

Strain SMA was grown in a large culture (100 ml) and DNA was extracted as described previously (Gasparich et al., 1993). The DNA was diluted in 1 x SSC buffer for a final sodium concentration of 0·195 M. This sample was analysed (with a 1 x SSC buffer blank) in a Cary Varian thermal spectrophotometer equipped with software to determine the melting temperature and the G + C content was determined (Carle et al., 1983). This process was repeated several times and Escherichia coli DNA in 1 x SSC buffer was used as a control. The G + C base content of strain SMA genomic DNA was measured as 21·56, 25·12 and 25·29 mol% (mean 24 ± 1 mol%).

**Phylogenetic analysis**

ATCC accession numbers and GenBank accession numbers for 16S rRNA gene sequences used in this study are indicated in Fig. 2(a). Sequences were aligned using CLUSTAL W (Thompson et al., 1994) and then aligned manually in MACCLADE (Maddison & Maddison, 1992). A 1000 replicate bootstrap analysis was performed using a heuristic search and the tree bisection reconnection parsimony algorithm from PAUP (Fig. 2a) and neighbour-joining analysis using the Jukes–Cantor model for substitutions (Fig. 2b) (version 4.0b; Swofford, 1998). In all analyses, strain SMA consistently grouped with the group VIII subgroups.

This is the first taxonomic and diagnostic description of a spiroplasma isolated from a lepidopteran larva. The white satin moth (L. salicis) is recognized as an invasive alien species and a major pest that defoliates mainly poplar trees (Populus spp.) but is spreading damage to other trees like trembling aspen, black cottonwood and sometimes willow (Humphreys, 1996). Isolation and characterization of strain SMA may contribute to attempts to establish biological control of satin moth larvae, which will be useful in efforts to curb deforestation.

As all the tests needed for the characterization of a novel spiroplasma species, as required by the International Committee on Systematics of Prokaryotes Subcommittee on the Taxonomy of Mollicutes, have been fulfilled, we propose a novel species *Spiroplasma leucomae* sp. nov.

**Description of Spiroplasma leucomae** sp. nov.

*Spiroplasma leucomae* [leu.co’mae. N.L. gen. fem. n. leucomae of Leucoma, systematic genus name of the white

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**Fig. 2.** Phylogenetic trees showing the position of *S. leucomae* strain SMA among representatives of the genera *Spiroplasma* and *Mycoplasma*. The 16S rRNA gene sequence of *S. leucomae* strain SMA was included in a dataset described by Gasparich et al. (2004). *Mycoplasma pneumoniae* ATCC 15531 was used as an outgroup strain. (a) The tree shows a bootstrap parsimony analysis of the dataset, which was resampled 1000 times. Bootstrap percentage values are given at nodes. ATCC accession numbers are shown next to the organism names and GenBank accession numbers for the 16S rRNA gene sequences used are given in parentheses. (b) Tree constructed by neighbour-joining analysis with the Jukes-Cantor substitution model. Bar, 0·01 substitutions per site. Strain and sequence details are shown in (a) with the exception of strain N525 [not included in (a)].
type strain of strain SMAT (Lepidoptera: Lymantriidae), the source of the type strain.

Cells are filamentous, helical and motile, and are approximately 150 nm in diameter. They pass freely through filters with 450 and 220 nm pores, but do not pass through filters with 100 nm pores. The cells lack true cell walls. Chemoorganotrophic. Acid is produced from glucose and arginine is catabolized. Does not utilize urea. Serologically distinct from previously established Spiroplasma species, groups and subgroups. The type strain was isolated from a fifth instar white satin moth (Leucoma salis) larva. Pathogenicity for these moth larvae is not known. The G+C content of the DNA is 24±1 mol% as determined by the melting temperature method.

The type strain is strain SMA\(^T\) (= ATCC BAA-521\(^T\) = NBRC 100392\(^T\)).

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### References


