Spiroplasma floricola, a New Species Isolated from Surfaces of Flowers of the Tulip Tree, *Liriodendron tulipifera* L.

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Spiroplasma strains 23-6 and 27-31 from flowers of *Liriodendron tulipifera* L. (tulip tree) were indistinguishable from one another but were distinct from *Spiroplasma citri* and from members of serogroups other than the serogroup which contains *S. citri*. These two strains shared close to 100% deoxyribonucleic acid homology with one another, but only low homology (<5%) with *S. citri* and other *Spiroplasma* strains. The acrylamide gel electrophoretic patterns of the cellular proteins of strains 23-6 and 27-31 appeared to be identical to each other, but they were distinct from the patterns of *S. citri* and various other strains. On the basis of serological and biochemical characteristics, we propose that strains 23-6 and 27-31 belong to a new species, *Spiroplasma floricola*, with strain 23-6 (= ATCC 29989) as the type strain. This new species grew well at 30 and 37°C, in contrast to *S. citri*, which grows poorly if at all at 37°C. During log-phase growth in broth medium, *S. floricola* cells occurred as motile, helical, cell wall-less filaments 0.15 to 0.2 µm in diameter and 2 to about 5 µm long. Classical "fried-egg" *Mycoplasma*-type colonies were not observed on 1% agar medium. Instead, colonies had granular centers and diffuse borders and contained abundant, motile, helical cells. Growth was stimulated by cholesterol and inhibited by digitonin. Tests for catabolism of glucose, fructose, mannose, and arginine were positive. Tests for phosphatase activity were negative. The guanine-plus-cytosine contents of the deoxyribonucleic acids of strains 23-6 and 27-31 were close to 25 mol%.

Spiroplasmas were first recognized in and cultivated in vitro from diseased plants (13, 14, 16, 29). It is now apparent that spiroplasmas are widespread in nature and that some strains associated with plants probably do not cause plant disease (7, 9, 10, 12, 22). Other *Spiroplasma* strains are associated with diseases in insects (6, 39, 41) or are found in apparently healthy insects (17; R. E. McCoy, J. H. Tsai, and D. L. Thomas, *Phytopathol. News* 12: 217, 1978) and ticks (2, 5, 25, 30, 35, 36, 43). Several distinct species are doubtlessly represented by the diverse strains now in culture in vitro, but only one species, *Spiroplasma citri*, has been recognized formally thus far (28).

Among the spiroplasmas from varied habitats are strains 23-6 and 27-31, which were isolated from surfaces of flowers of the tulip tree (*Liriodendron tulipifera* L.) (9, 10, 12). Triply cloned strain 23-6 was shown to be serologically distinct from the honey bee *Spiroplasma* (strain AS 576) and from *S. citri*, and Davis suggested that strain 23-6 represented a new species (9). Subsequently, a scheme for the classification of *Spiroplasma* strains into serogroups and serological

subgroups on the basis of membrane antigen reactivity was proposed (12). Estimations of deoxyribonucleic acid (DNA) base compositions and DNA-DNA homologies confirmed that the classification scheme reflected phylogenetic relationships (18) and supported the proposal (12) that the serogroups represented separate species. Strains 23-6 and 27-31 were serologically indistinguishable from one another and comprised a single subgroup in a major serogroup that exhibited no cross-reactions with other groups, and Davis et al. suggested that these two strains represented a single distinct species (12). In this paper we present gel electrophoretic analyses of cellular proteins and describe morphological, physiological, and biochemical properties of strains 23-6 and 27-31 which support our contention that these two strains belong to a new species.

MATERIALS AND METHODS

***Spiroplasma* strains.** Strains 23-6 and 27-31 were isolated from surfaces of flowers of *L. tulipifera* L. (9). Both of these strains were triply cloned by a procedure described previously (9). Strain 23-6 was deposited

with the American Type Culture Collection as strain ATCC 29989. Other *Spiroplasma* strains used in this study included *S. citri* Maroc R8A2 (=ATCC 27556), *S. citri* C189 (=ATCC 27665), strains AS576 (=ATCC 29416) and G9, which were isolated from honey bees, and strains G1, SR9, and SR3 (=ATCC 33095), which were isolated from flowers (10, 12; R. E. Davis, *Phytopathol. News* 12:PO-7, 1978; R. E. Davis, J. F. Worley, T. B. Clark, and M. Moseley, *Proc. Am. Phytopathol. Soc.* 3:304, 1976). *S. citri* Maroc R8A2 and *S. citri* C189 were obtained from the American Type Culture Collection. *Spiroplasma* strain PPS1 was kindly furnished by R. E. McCoy (University of Florida Agricultural Research Center, Fort Lauderdale), who isolated it from flowers of *Calliandra haematocephala* (powder puff) (22). The remaining *Spiroplasma* strains were isolated in our laboratory in Beltsville, Md. The *Acholeplasma laidlawii* strain used was ATCC 23206.

Culture medium. Unless otherwise indicated, the spiroplasmas were cultivated in medium DSM4, which was similar to the medium of Liao and Chen (3, 20) and contained (per 100 ml) 1.5 g of PPLO broth base (Difco Laboratories, Detroit, Mich.), 8.0 g of sucrose, 10 ml of inactivated (30 min at 56°C) horse serum (Microbiological Associates, Bethesda, Md.), and 2.5 mg of phenol red, as described previously (9). Solid medium contained 1.0 g of Noble agar (Difco) per 100 ml. All of the ingredients except horse serum were dissolved in distilled water to give a total volume of 90 ml of basal medium, the pH was adjusted to 7.4, and the basal medium was sterilized by autoclaving for 15 min at 121°C. Sterile horse serum was added aseptically to the autoclaved and cooled basal medium.

Morphology and ultrastructure. Dark-field optical microscopy was performed with a Zeiss model II photomicroscope. For ultrathin section electron microscopy, colonies in agar medium were treated by a method previously described (42) for microscopy of infected plant tissues. Freeze-etch electron microscopy was performed as described elsewhere (31).

Ultrafiltration. Samples from a 20-h-old culture of strain 23-6 grown at 31°C were filtered through sterile membrane filters (pore size, 800, 650, 450, 300, or 200 nm; Millipore Corp., Bedford, Mass.) in Swinney hypodermic adapters. Filtration was performed under conditions that uniformly gave a pressure of 116 g/cm² at the filter surface. The titers of the spiroplasmas in the filtrates were determined by duplicate plating of serial 10-fold dilutions on solid agar medium.

Influence of serum on growth. The influence of serum concentration on growth was determined in broth media containing 10% yeast extract (Flow Laboratories, Inc., Rockville, Md.) and 0, 0.1, 0.5, 1, 2, 5, 10, or 20% inactivated horse serum. Portions of these media were seeded with a log-phase culture of strain 23-6 diluted with serum-free basal medium to give an initial titer of 3.4 log colony-forming units per ml in each medium. After 16 and 26 h of incubation at 32°C, the *Spiroplasma* titer at each serum concentration was determined by duplicate plating onto agar medium.

Growth inhibition by digitonin. Inhibition of growth by digitonin was determined in broth and agar

media. In broth tests modified from the method of Razin and Schafer (26), the serum-free base of broth medium DSM4 was supplemented with 0.5% bovine serum albumin (fraction V; Sigma Chemical Co., St. Louis, Mo.), palmitic acid (10 µg/ml), and cholesterol (5 or 10 µg/ml). Stock cholesterol was prepared as an ethanolic solution (27). Portions (5 ml) of medium containing from 0 to 100 µg of digitonin per ml were each seeded with 0.1 ml of a 24-h-old culture of strain 23-6 (diluted in medium containing 10 µg of cholesterol per ml) and incubated for 24 to 72 h at 31°C. A change in color of phenol red to yellow indicated growth; the presence of helical *Spiroplasma* cells was confirmed by dark-field optical microscopy. *A. laidlawii* ATCC 23206 was used as a control. In tests on solid agar medium (15), medium DSM4 supplemented with 5 or 10% inactivated horse serum was used. Zones of inhibition were recorded after 5 to 7 days of incubation at 25, 31, or 37°C. *S. citri* strain Maroc R8A2 and *A. laidlawii* ATCC 23206 were used as controls, and *Spiroplasma* strain SR3 was used as an additional test organism in the tests on solid agar medium.

Influence of cholesterol on growth. The influence of cholesterol on the growth of *Spiroplasma* strain 23-6 was determined by a broth method (27). Portions (50 ml) of medium (serum-free DMS4 basal medium supplemented with 10% fresh yeast extract and 0.5% bovine serum albumin with or without 10 µg of palmitic acid per ml, 0.01% Tween 80, and varying amounts of cholesterol) were each seeded with 0.5 ml of a 1:1,000 dilution (unsupplemented serum-free basal medium was used as the diluent) of a 24-h-old culture of *Spiroplasma* strain 23-6 grown at 32°C in broth medium DSM4 containing serum (9). After 72 h of incubation at 32°C, *Spiroplasma* cell protein was determined (27) by the method of Lowry et al. (21).

Catabolism of sugars and arginine and phosphatase activity. Tests of sugar catabolism were performed in medium D187C, which contained (per 100 ml) 2.0 g of tryptose (Difco), 0.5 g of NaCl, 2.5 mg of phenol red, and a serum supplement. The serum supplement was either 5% horse serum (GIBCO Laboratories, Grand Island, N.Y.) or 1% PPLO serum fraction (Difco). This medium was supplemented with 0.5% glucose, 0.5% mannose, 0.5% fructose, 0.4% L-arginine, or (in the case of strain AS576) 0.4% arginine plus 0.01% fructose (after Townsend [33]), which was sterilized by filtration (pore size, 220 nm; Millipore). The final pH's of the resulting media were 7.1 to 7.3. Portions (2 ml) of medium were each seeded with 0.05 ml of a 1:1,000 dilution (diluted in medium D187C without substrates or serum) of a 24-h-old culture of *Spiroplasma* cells, and these cultures were incubated for up to 7 days at 31°C. A shift in the color of the pH indicator to yellow (acid in the case of the sugars) or to an alkaline reaction (deeper red or purple hue in the case of arginine or arginine plus fructose) was recorded as positive. Unseeded media and seeded media without substrate were used as controls.

Phosphatase activity was measured at 31 and 37°C by the method of Williams and Wittler (38) in solid 1% agar PPLO broth base medium (without phenol red) containing 5% inactivated horse serum and 0.01% phenolphthalein diphosphate. Plates were seeded in

triplicate with 0.1 ml of each of several 10-fold dilutions of a 20-h-old culture and were incubated at 31 or 37°C. At 5, 7, and 9 days, one plate containing each dilution of inoculum with colonies was flooded with 5 N NaOH.

Polyacrylamide gel electrophoresis of cellular proteins. Cells were grown in broth medium for 20 h at 31°C, harvested by centrifugation, and washed in a 12% sucrose solution. Protein was determined by the method of Lowry et al. (21). Proteins were solubilized by the method of Daniels and Meddins (8), and electrophoresis was performed on 7% polyacrylamide gel slabs in a vertical gel electrophoresis apparatus (Aquebogue Machine Shop, Aquebogue, N.Y.).

RESULTS

Morphological, cultural, and biochemical properties. Strains 23-6 and 27-31 were similar in morphological, cultural, and biochemical properties. We have described several characteristics of strain 23-6 previously (9, 10). Cultures of strains 23-6 and 27-31 in broth medium contained motile, helical, filamentous cells during log-phase growth. Few or no rounded bodies were observed; no branched cells were seen. Helical cells were 0.15 to 0.2 μm in diameter and 2 to about 5 μm long in log-phase cultures (9). Freeze-etch electron microscopy revealed the particulate nature of the protoplasmic fracture faces of membranes of the *Spiroplasma* cells and showed a narrow, smooth band with few or no membrane-associated particles (Fig. 1). These characteristics are similar to those previously reported for the corn stunt *Spiroplasma* (R. L. Steere and R. E. Davis, *Phytopathology* 62:672, 1972).

Growth occurred at 22 to 37°C, with optimal growth at about 34°C (9). This contrasted significantly with *S. citri* strains, which have been reported (28) to grow poorly, if at all, at 25 and 37°C. Broth cultures developed marked turbidity. In ordinary broth medium, individual cells exhibited flexional and rotational motility, with little or not forward or reverse progress. In broth medium of elevated viscosity, the helical cells exhibited considerable translational locomotion, as reported previously (9, 10).

"Fried-egg" colonies were not observed on media solidified with agar. Instead, colonies were spreading and diffuse (9). Abundant, motile, helical *Spiroplasma* cells were seen in colonies examined by dark-field optical microscopy. Electron microscopy of ultrathin sections of colonies in agar revealed the three-layered limiting membrane of the *Spiroplasma* cells (Fig. 2) but failed to indicate the presence of any cell wall, envelope, second membrane, periplasmic fibrils, or flagellar structure (9).

Table 1 shows the results from the tests for catabolism of sugars and arginine. These results were similar whether media were supplemented with serum or with PPLO serum fraction. Strains 23-6 and 27-31 were strongly positive for catabolism of glucose, fructose, mannose, and arginine. Strains SR3 and SR9 were positive for catabolism of glucose, fructose, mannose, and arginine. Strain AS576 was positive for catabolism of glucose, fructose, and arginine but negative for catabolism of mannose. These findings agree with results reported previously (10). Strain PPS1 was strongly positive for catabolism

FIG. 1. Stereo electron micrographs of freeze-fractured and etched cells of *Spiroplasma* strain 23-6. Note narrow smooth band containing few or no membrane-associated particles (arrows). Bar = 200 nm. (From R. L. Steere.)

FIG. 2. Electron micrograph of ultrathin section of *Spiroplasma* strain 23-6 cells in colonies produced on agar medium. Bar = 200 nm.

TABLE 1. Catabolism of glucose, fructose, mannose, and arginine by *Spiroplasma* strains 23-6, 27-31, SR3, and SR9 from flowers of *L. tulipifera*, strain AS576 from honey bees, and strain PPS1 from flowers of *C. haematoccephala*

<i>Spiroplasma</i> strain	Catabolism of: ^a			
	Glucose	Fructose	Mannose	Arginine
23-6	+	+	+	+
27-31	+	+	+	+
SR3	+	+	+	+
SR9	+	+	+	+
AS576	+	+	—	+
PPS1	+	+	W	—

^a Media contained 0.5% glucose, 0.5% fructose, 0.5% mannose, or 0.4% arginine, with the exception of the arginine medium for strain AS576, which contained 0.4% arginine and 0.01% fructose.

^b +, Positive; —, negative; W, weakly positive.

of glucose and fructose, weakly positive for catabolism of mannose, and negative for catabolism of arginine. The lack of arginine hydrolysis by strain PPS1 is in agreement with the results reported by McCoy et al. (22).

Both strain 23-6 and strain 27-31 were isolated originally in media lacking antibiotics (9), and they have been maintained in media free of antibiotics for at least 25 subcultures with no evidence of reversion to bacterial forms. They were strongly inhibited by tetracycline antibiotics and erythromycin but were relatively resistant to streptomycin (10, 11). Growth was not inhibited in media containing 10,000 U of penicillin G per ml. Tests for phosphatase activity were negative. The guanine-plus-cytosine con-

tents of the DNAs of these strains have been reported to be close to 25 mol% (18).

Ultrafiltration. When filtered through membrane filters with average pore diameters of 800, 650, 450, 300, and 220 nm, a triply cloned culture of strain 23-6 containing an estimated 6.7×10^8 colony-forming units per ml yielded 4.8×10^8 , 3.8×10^8 , 3.7×10^8 , 3.6×10^8 , and 2.8×10^8 colony-forming units per ml, respectively.

Influence of serum on growth. Table 2 shows the results of a representative experiment in which we tested the influence of horse serum on the growth of *Spiroplasma* strain 23-6 in broth medium. Little or no growth was observed in medium containing no added serum, but growth was stimulated in medium containing as little as 0.1% horse serum supplement.

Growth inhibition by digitonin. Growth was inhibited by digitonin in all tests, a result consistent with a requirement for sterol (15, 26). In broth tests, the minimal inhibitory concentration of digitonin depended upon the inoculum concentration of *Spiroplasma* cells and the duration of incubation before the results were read (Table 3). Thus, the growth of strain 23-6 was inhibited at concentrations of digitonin of 15 to 35 μ g/ml in media containing 10 μ g of cholesterol per ml (Table 3). Under the same conditions *A. laidlawii* ATCC 23206 was relatively resistant to digitonin; for example, when test results were read after 48 h of incubation, strain 23-6 was inhibited by 30 μ g of digitonin per ml, but growth of *A. laidlawii* ATCC 23206 was evident at 100 μ g of digitonin per ml, the highest concentration tested. In a separate test, growth of strain 23-6 was inhibited by digitonin at concentrations of 20 and 30 μ g/ml in media containing 5 and 10 μ g of cholesterol per ml, respectively, when the

TABLE 2. Influence of serum on the growth of *Spiroplasma* strain 23-6 in broth medium at 32°C^a

% of horse serum	Titer (log CFU/ml) after incubation for: ^b	
	16 h	26 h
0	3.1	3.8
0.1	5.2	7.0
0.5	5.7	7.6
1	5.7	7.8
2	5.9	8.0
5	5.9	8.3
10	5.9	8.3
20	5.7	8.2

^a The medium contained (per 100 ml) 1.5 g of PPLO broth base (Difco), 8.0 g of sucrose, 2.5 mg of phenol red, 10 ml of fresh yeast extract (Flow), and 0 to 20 ml of inactivated (56°C, 30 min) horse serum.

^b CFU, Colony-forming units. The titer at zero time was 3.4 log colony-forming units per ml.

results were read after 5 days of incubation at 31°C.

Table 4 shows the zones of inhibition of growth on solid agar medium containing 10% serum. Colony formation by flower *Spiroplasma* strains 23-6 and SR3 and by *S. citri* Maroc R8A2 was inhibited strongly by digitonin at the growth-permitting temperatures of 25, 31, and 37°C. *A. laidlawii* ATCC 23206 was inhibited only slightly (zone diameter, ≤ 1 mm) under these conditions.

Influence of cholesterol on growth. Growth was minimal in serum-free broth medium supplemented with bovine serum albumin or with bovine serum albumin, palmitic acid, and Tween 80 but was enhanced by increasing concentrations of cholesterol (Table 5), in agreement with characteristics described previously (32) for the family *Spiroplasmataceae*.

TABLE 3. Inhibition of *Spiroplasma* strain 23-6 growth by digitonin in broth medium^a

Titer of <i>Spiroplasma</i> cells at start of test (CFU/ml) ^b	Minimal inhibitory concentration of digitonin (μ g/ml) after incubation at 31°C for:	
	24 h	72 h
3.6×10^6	≤ 25	≤ 35
3.6×10^5	20	30
3.6×10^4	≤ 20	30
3.6×10^3	≤ 15	30

^a The medium contained (per 100 ml) 1.5 g of PPLO broth base (Difco), 8.0 g of sucrose, 2.5 mg of phenol red, 0.5% bovine serum albumin (fraction V), 10 μ g of palmitic acid per ml, and 10 μ g of cholesterol per ml.

^b CFU, Colony-forming units. Broth medium was seeded with a 24-h-old culture of *Spiroplasma* diluted with sterile broth to give initial titers of 3.6×10^3 to 3.6×10^6 colony-forming units per ml in media containing digitonin at concentrations of 0 to 50 μ g/ml.

TABLE 4. Inhibition by digitonin of growth of flower *Spiroplasma* strains 23-6 and SR3, of *S. citri* (Maroc R8A2), and of *A. laidlawii* ATCC 23206 on agar medium at 25, 31, and 37°C^a

Strain	Zone of inhibition (mm) at the following incubation temp:		
	25°C	31°C	37°C
23-6	9.0	8.5	9.5
SR3	8.5	8.0	ND ^b
<i>S. citri</i> Maroc R8A2	ND	14.0	ND
<i>A. laidlawii</i> ATCC 23206	1.0	<1.0	<1.0

^a The medium contained (per 100 ml) 1.5 g of PPLO broth base (Difco), 8.0 g of sucrose, 2.5 mg of phenol red, 1.0 g of Noble agar (Difco), and 10 ml of inactivated (56°C, 30 min) horse serum. Results were recorded after 7 days of incubation.

^b ND, not done.

Serological reactions. The results of serological tests of growth inhibition, organism deformation, and ring interface precipitin and microprecipitin tests in which purified membrane preparations were used as test antigens have been reported previously (9, 12). We observed no cross-reactions between strain 23-6 and any of the *Spiroplasma* strains listed in Table 6 when antigens or antisera to the latter or both were used (9, 12). Strains 23-6 and 27-31 were indistinguishable by growth inhibition and organism deformation tests but were distinct from the other *Spiroplasma* strains examined, including strains of *S. citri*.

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis of cellular proteins from strains 23-6 and 27-31 gave identical patterns that were distinct from the pattern of strain SR3 (Fig. 3). The polyacrylamide gel electrophoresis protein pattern of strain 23-6 was also distinct from the patterns of *S. citri* and other *Spiroplasma* strains from plants and insects (Fig. 4).

DISCUSSION

Strains 23-6 and 27-31 from the surfaces of flowers clearly belong to the genus *Spiroplasma*, based on morphological, ultrastructural, growth requirement, and other characteristics described previously (9, 10, 17, 18) and evaluated more extensively here. We propose that strains 23-6 and 27-31 represent a single new species of *Spiroplasma*, based on similarities in properties of these two strains and on their distinctness from *S. citri*, the only *Spiroplasma* species formally proposed previously (28). That certain other strains now in cultivation may represent additional new species is strongly suggested by related work. Indeed, during the past several years investigations by several groups of researchers

TABLE 5. Influence of cholesterol on growth of *Spiroplasma* strain 23-6 in serum-free broth medium

Concn of cholesterol (μ g/ml)	Concn of cell protein ^a
0 ^b	0.03
0 ^c	0.03
0.2	0.16
1	0.62
5	0.86
10	0.74
20	0.75

^a Expressed in milligrams of protein per 50 ml of culture.

^b Serum-free medium supplemented with 0.5% bovine serum albumin.

^c Serum-free medium was supplemented with 0.5% bovine serum albumin, 10 μ g of palmitic acid per ml, and 0.01% Tween 80.

TABLE 6. *Spiroplasma* strains used in serological tests

Serogroup	<i>Spiroplasma</i> species or strain designation	Source from which originally isolated	Reference(s)
I (A) ^a	<i>S. citri</i> Maroc R8A2 (=ATCC 27556)	Citrus (<i>Citrus sinensis</i> (L.) Osbeck)	14, 23, 24
I (B)	AS576 (=ATCC 29416)	Honey bee	Davis et al. ^b
I (B)	G1	Surface of <i>Bidens pilosa</i> L. flower ^c	Davis ^d
I (B)	BW	Surface of basswood flower (<i>Tilia americana</i> L.) ^c	10
I (C)	1-747 (corn stunt; =ATCC 29051)	Corn (<i>Zea mays</i> L.)	4
II	23-6 (=ATCC 29989)	Surface of <i>L. tulipifera</i> flower ^c	8, 10, 11
II	27-31	Surface of <i>L. tulipifera</i> flower ^c	10, 11
III	SR3 (=ATCC 33095)	Surface of <i>L. tulipifera</i> flower ^c	10, 11
III	SR9	Surface of <i>L. tulipifera</i> flower ^c	8, 10, 11
IV	SMCA (suckling mouse cataract spiroplasma; =ATCC 29335) ^c	Rabbit tick	26, 27

^a Letters in parentheses are serological subgroups.^b Davis et al., Proc. Am. Phytopathol. Soc. 3:304, 1976.^c These spiroplasmas were presumably present in nectar.^d Davis, Phytopathol. News 12:PO-7, 1978.^e Growth inhibition test only. FIG. 3. Electrophoretic patterns of proteins from *Spiroplasma* strains 27-31 (lane A), SR3 (lane B), and 23-6 (lane C).

FIG. 4. Electrophoretic patterns of proteins from strains 1-747 (lane A), G1 (lane B), Maroc R8A2 (lane C), AS576 (lane D), C189 (lane E), and 23-6 (lane F).

have shown that the cultured spiroplasmas comprise several types distinctly different from one another and from *S. citri* (1, 4, 9, 10, 12, 19, 22-24, 34-36, 40). Spiroplasmas isolated from flowers have been important elements in some of these investigations.

Spiroplasmas that occur on surfaces of flowers (presumably in nectar) were first isolated in culture from flowers of *L. tulipifera* L. (9). Shortly thereafter, other isolates were recovered from flowers of *Magnolia grandiflora* L. (7) and *C. haematocephala* (22). The isolates from tulip tree flowers included strains 23-6, 27-31, SR3, and SR9 (9, 12). Two new separate species were thought to be represented among these four

strains (9, 10, 12). In a scheme for the classification of *Spiroplasma* strains into serogroups and serological subgroups based on results from tests on membrane antigen reactivity (12), strains 23-6 and 27-31 were serologically indistinguishable and were placed in a single major serogroup (serogroup II). Flower *Spiroplasma* strains SR3 and SR9 were placed in another group (serogroup III), to which additional strains from flowers and insects have been added more recently (25a, 34, 37). The lack of cross-reactions between serogroups II and III and

between these groups and a separate group (serogroup I) containing *S. citri* was believed to be taxonomically significant (12). Work by others has further shown the feasibility of serological grouping of *Spiroplasma* strains (40; J. G. Tully, D. L. Williamson, D. L. Rose, and R. F. Whitcomb, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, G8, p. 83). For example, it has been shown that *Spiroplasma* strains OBMG and BNR1, which were isolated by T. B. Clark from magnolia and tulip tree flowers, respectively, and were found by polyacrylamide gel electrophoresis analyses of proteins to be identical to strain 23-6 (M. J. Daniels, personal communication), are distinct from *Spiroplasma* strains in other serological groups (17, 40).

The results from polyacrylamide gel electrophoresis studies of cellular proteins and DNA homology experiments among *Spiroplasma* strains agree well with the identifications and grouping based on serological reactions (1, 4, 17, 18, 23, 24; M. J. Daniels, personal communication). For example, strains 23-6 and 27-31 have identical cellular protein electrophoretic patterns (see above) but are distinct from *S. citri* and from *Spiroplasma* strains belonging to other serogroups (Daniels, personal communication). Furthermore, strains 23-6 and 27-31 share close to 100% DNA homology with one another, but DNA homology between these strains and *S. citri* and other spiroplasmas was estimated at $\leq 5\%$ (18). *Spiroplasma* groups and subgroups with strains separated on the basis of serology had the same identities as the groups and subgroups identified by reciprocal DNA homology studies (18). In this work, the guanine-plus-cytosine contents of the strain 23-6 and 27-31 DNAs were found to be close to 25 mol%, a value approximating the value for *S. citri* DNA (26 mol%) (1, 28) but distinct from the value of 29 mol% reported for the DNAs from the suckling mouse cataract agent (1, 4) and flower *Spiroplasma* strains SR3 and SR9 (18). In a separate study (17), DNAs from strains 23-6, OBMG, and BNR1 were also found to contain about 25 mol% guanine plus cytosine and to share no homology with *S. citri*. The available data are consistent with the conclusion that *Spiroplasma* strains 23-6 and 27-31 are very similar or identical to one another and are distinct from *S. citri* and members of other serogroups.

In accordance with criteria proposed by the International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of *Mollicutes* for separation of species (32), the distinction of strains 23-6 and 27-31 from *S. citri* by serology, gel electrophoresis of cellular proteins,

and DNA homology indicates that strains 23-6 and 27-31 belong to a single new species of *Spiroplasma*.

Based on the findings discussed above, we propose that *Spiroplasma* strains 23-6 and 27-31 be placed in a new species, for which we propose the name *Spiroplasma floricola* (L. n. *flora* flower; L. v. *colo* dwell M. L. n. *floricola* flower dweller). Strain 23-6 (=ATCC 29989) is the type strain. This new species occurs in flower nectar and is characterized by motile, helical, wall-less cells 0.15 to 0.2 μm in diameter and 2 to about 5 μm long during log-phase growth in DSM4 broth medium, by inhibition of growth by digitonin, by enhancement of growth by increasing concentrations of cholesterol, and by a DNA base composition of about 25 mol% guanine plus cytosine (Table 7). The type strain and strain 27-31 are capable of growth at 22 to 37°C and grow rapidly at 30 to 37°C, yielding turbid cultures in DSM4 broth medium and diffuse, spreading colonies on agar medium. These two strains catabolize glucose, fructose, mannose, and arginine but lack phosphatase activity.

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TABLE 7. Summary of several biological and biochemical properties of *S. floricola*

Characteristic	Observation
Cellular morphology	Helical
Motility	Rotational, flexional; translational in semisolid and viscous media
Catabolism of glucose	+
Catabolism of fructose	+
Catabolism of mannose	+
Catabolism of arginine	+
Phosphatase activity	—
Growth inhibition by digitonin	+
Growth enhanced by cholesterol	+
Culture turbidity	Heavy
Colonies on agar medium	Diffuse, spreading
Growth at 25°C	+
Growth at 31°C	+
Growth at 37°C	+
Base composition of DNA	25 mol% guanine plus cytosine
DNA homology with <i>S. citri</i>	<5%
Type strain	23-6 (=ATCC 29989)
Source of type strain	Surface (presumably nectar) of flower of <i>L. tulipifera</i> L.

ance of L. K. Basciano, E. Erbe, M. H. Hale, B. L. Nivera, and C. J. Teuton in this work.

REPRINT REQUESTS

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