Erigeron Witches'-Broom Phytoplasma in Brazil Represents New Subgroup VII-B in 16S rRNA Gene Group VII, the Ash Yellows Phytoplasma Group

Thereza S. L. Barros, USDA-ARS, Molecular Plant Pathology Laboratory, Beltsville, MD 20705, USA, and Laboratório de Virologia e Microscopia Eletrônica, Departamento de Biologia Celular, Universidade de Brasília, Brasília, DF, 70919-970, Brazil; **Robert E. Davis,** USDA-ARS, Molecular Plant Pathology Laboratory, Beltsville, MD; **Renato O. Resende,** Laboratorio de Virologia e Microscopia Eletrônica, Departamento de Biologia Celular, Universidade de Brasília, Brasília, DF, 70919-970, Brazil; and **Ellen L. Dally,** USDA-ARS, Molecular Plant Pathology Laboratory, Beltsville, MD

ABSTRACT

Barros, T. S. L., Davis, R. E., Resende, R. O., and Dally, E. L. 2002. Erigeron witches'-broom phytoplasma in Brazil represents new subgroup VII-B in 16S rRNA gene group VII, the ash yellows phytoplasma group. Plant Dis. 86:1142-1148.

A previously undescribed phytoplasma, Erigeron witches'-broom phytoplasma, was detected in diseased plants of *Erigeron* sp. and *Catharanthus roseus* exhibiting symptoms of witches'-broom and chlorosis in the state of São Paulo, Brazil. On the basis of restriction fragment length polymorphism (RFLP) analysis of 16S rDNA amplified in the polymerase chain reaction (PCR), Erigeron witches'-broom phytoplasma was classified in group 16SrVII (ash yellows phytoplasma group), new subgroup VII-B. Phylogenetic analysis of 16S rDNA sequences indicated that this phytoplasma represents a new lineage that is distinct from that of described strains of ash yellows phytoplasma. Erigeron witches'-broom phytoplasma is the first member of the ash yellows phytoplasma group to be recorded in Brazil.

Additional keywords: MLO, mycoplasmalike organism, phylogeny

Phytoplasmas (previously mycoplasmalike organisms [MLOs]) are unique cell-wall-less, prokaryotic pathogens of plants (17). Because it has not been possible to isolate and characterize any phytoplasma in pure culture in vitro, the detection and identification of phytoplasmas was long based upon biological characteristics of the pathogens and associated plant diseases (17). Molecular methods afforded new approaches that have led to discoveries of diverse phytoplasmas and to the construction of a comprehensive scheme for phytoplasma identification and classification. On the basis of restriction fragment length polymorphism (RFLP) analyses of 16S rDNA, phytoplasmas have been classified into groups and subgroups, each distinct group representing at least one putative phytoplasma species (13,17,18,22). Phylogenetic analyses have revealed that phytoplasmas descended from gram-positive bacterial ancestors and form a monophyletic clade in the class Mollicutes (13,20,24,29,30). New understanding of phytoplasma diversity and phylogenetic relationships, the discovery of diverse phytoplasma lineages, and a provisional taxonomy involving descriptions of "*Candidatus* Phytoplasma species" have emerged from applications of molecular methods (6,11,22,28,35,36).

In South America, phytoplasmas belonging to four major groups (groups 16SrI, 16SrIII, 16SrIX, and 16SrXV, respectively), representing at least four putative species, have been characterized (1,3,7,22,23). Phytoplasmas in South America that belong to group 16SrIII (X-disease phytoplasma group) and group 16SrXV (hibiscus witches'-broom phytoplasma group) are distinct from phytoplasmas described elsewhere, and hibiscus witches'-broom phytoplasma has been described as a distinct "*Candidatus* Phytoplasma species", "*Ca.* P. brasiliense" (22,23). Previously we hypothesized that unique ecology and geographic separation provided conditions favorable for the divergence, in South America, of phytoplasma lineages from those found in other regions of the world

Corresponding author: Robert E. Davis
E-mail: davisr@ba.ars.usda.gov

GenBank Accession numbers of DNA sequences: AY034608, AF411592

Accepted for publication 23 May 2002.

Publication no. D-2002-0815-01R

Table 1. GenBank accession numbers of phytoplasmal and *Acholeplasma laidlawii* 16S rRNA gene sequences used in this study

Phytoplasma (strain)	Accession no.
Erigeron witches'-broom (EriWB)	AY034608
Rio das Pedras witches'-broom (RPWB)	AF411592
Apple proliferation (AT)	X68375
Aster yellows (AY)	AF322644
Bermuda grass white leaf (BGWL)	AF248961
Brinjal little leaf (BLL)	X83431
"*Candidatus* Phytoplasma australiense" (AUSGY)	L76427
"*Ca.* P. brasiliense" (HibWB)	AF147708
"*Ca.* P. fraxini" (ash yellows, AshY1^T)	AF09209
"*Ca.* P. fraxini" (ash yellows, AshY3)	AF105315
"*Ca.* P. fraxini" (ash yellows, AshY5)	AF105316
"*Ca.* P. fraxini" (lilac witches'-broom, LWB3)	AF105317
"*Ca.* P. aurantifolia" (LimeWB)	U15442
"*Ca.* P. japonicum"	AB010425
Clover phyllody (CPh)	AF222065
Clover proliferation (CP)	L33761
Clover yellow edge (CYE-C)	AF175304
Coconut lethal yellows (LY)	U18747
Elm yellows (EY1)	AF122910
Loofah witches'-broom (LfWB)	AF248956
Mexican periwinkle virescence (MPV)	AF248960
"Fragaria multicipita" phytoplasma (MC)	AF190224
Peanut witches'-broom (PnWB)	L33765
Pigeon pea witches'-broom (PPWB)	U18763
Rice yellow dwarf (RYD)	D12581
Stolbur (STOL)	X76427
Tomato big bud (BB)	AF222064
Western X-disease (WX)	L04682
Acholeplasma laidlawii	M23932

(22). The results of the present study support this hypothesis and provide new knowledge about the diversity of phytoplasma lineages in South America. In this communication, we describe molecular characterization of a previously undescribed phytoplasma infecting *Erigeron* sp. in Brazil and report its phylogenetic relationship to phytoplasmas elsewhere, including its close relationship to a distinct phytoplasma group originally described in North America.

MATERIALS AND METHODS

Plant samples and reference phytoplasma strains. Symptomatic leaves of erigeron (*Erigeron* sp.) and periwinkle (*Catharanthus roseus* L. (G. Don)) were collected from naturally diseased plants exhibiting symptoms of witches'-broom disease in the state of São Paulo, Brazil. Ash yellows (AshY) phytoplasma strain AshY1^T (type strain of "*Candidatus* Phytoplasma fraxini" and member of group 16SrVII) and clover proliferation phytoplasma strain CP (member of group 16SrVI) (kindly supplied by I.-M. Lee) were used as reference strains in direct comparisons of RFLP patterns of 16S rDNA. The reference phytoplasmas were maintained by grafting in plants of *C. roseus* grown in an insect-proof greenhouse.

Amplification of DNA in the polymerase chain reaction (PCR). DNA for use as template in PCRs was extracted from symptomatic plant tissues as described previously by Prince et al. (27). The template DNA was further purified by the use of GeneCleanIII Kit (Q-BIOgene, Carlsbad, CA). Universal phytoplasma primer pairs, P1/P7 (33) and R16F2n/R16R2 (F2n/R2) (12), were used to prime amplification of phytoplasma 16S rDNA (16S rRNA gene) sequences in nested PCR assays. Products of PCRs primed by P1/P7 were diluted 1:50 with HPLC grade water (Sigma-Aldrich, St. Louis, MO) and used as templates in PCRs primed by F2n/R2. All reactions were performed in 0.5-ml PCR tubes, in a final volume of 50 µl of reaction mixture, as previously described (6). PCRs were carried out for 35 cycles using the following conditions: 1 min (3 min for first cycle) denaturation at 94°C, annealing for 2 min at 55°C, and primer extension for 3 min (10 min in final cycle) at 72°C. Negative controls consisted of reaction mixtures devoid of templates. PCR products were analyzed by electrophoresis through 1% agarose gel, staining with ethidium bromide, and visualization of DNA bands using a UV transilluminator. The DNA fragment size standard used was 1-kb ladder (Invitrogen/Life Technologies, Gaithersburg, MD).

RFLP analyses of amplified phytoplasma DNA. Products from the nested PCRs primed by F2n/R2 were analyzed by single restriction endonuclease digestion with *Alu*I, *Hha*I, and *Kpn*I (Invitrogen/Life Technologies), and with *Bfa*I, *Hae*III, *Hin*fI, *Hpa*II, *Mse*I, *Rsa*I, *Sau*3AI, *Taq*I, and *Tha*I (New England BioLabs, Beverly, MA), following instructions by the manufacturers. The products of digestion were analyzed by electrophoresis through a 5% polyacrylamide gel followed by staining with ethidium bromide and visualization of DNA bands with a UV transilluminator. The DNA fragment size standard used was a PhiX174 RF *Hae*III digest (Invitrogen/Life Technologies). The RFLP patterns of phytoplasma DNAs were compared with the RFLP patterns previously published (18). Group affiliations are designated according to the classification system of Lee et al. (18,19).

Cloning of PCR products and sequencing of DNA. Two phytoplasma DNA sequences, one from *Erigeron* sp. and one from *C. roseus*, that had been amplified in PCRs primed by P1/P7 were cloned in *Escherichia coli* using the TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. Both strands of the cloned 1.8-kbp DNA fragments of rDNA were sequenced by use of automated DNA sequencing. The sequences were assembled after a minimum of 2× sequencing coverage for each base position. The nucleotide sequences determined in this study were deposited in the GenBank data library (National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD). Other phytoplasmal and *Acholeplasma laidlawii* 16S rDNA sequences used in this study were obtained from GenBank (Table 1).

Sequence similarity, coefficient of similarity calculations, and putative restriction site analysis. The 16S rDNA sequence similarities between strains were evaluated after alignments were generated by using the MegAlign option of DNASTAR program (DNASTAR, Inc., Madison, WI). Coefficients of similarity (F) of 16S rDNA were calculated on the basis of putative restriction site maps generated by using the DNASTAR program MapDraw option. F was calculated, according to Nei and Li (25), as $F = 2N_{xy}/(N_x + N_y)$, where x and y are the strains of two given phytoplasmas, N_x and N_y are numbers of fragments resulting from digestion of 16S rDNA by 12 enzymes in strains x and y respectively, and N_{xy} is the number of fragments shared by the two strains.

Phylogenetic analysis. 16S rRNA gene sequences (1.2 kbp in size, representing the sequence between annealing sites of primer pair F2n/R2) from EriWB phytoplasma and 26 other phytoplasma strains representing fifteen 16S rRNA phytoplasma

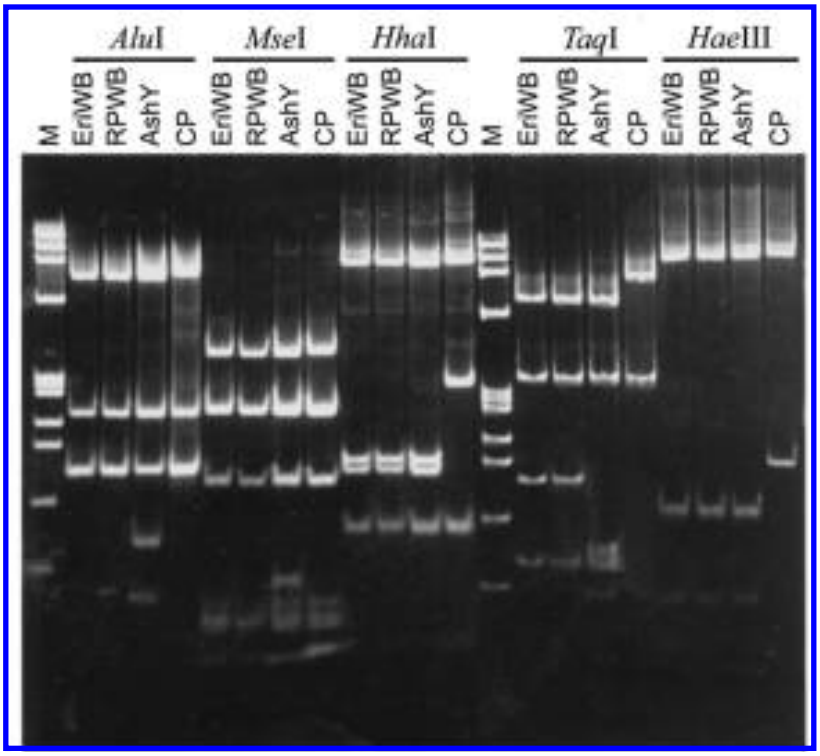

Fig. 1. Restriction fragment length polymorphism (RFLP) analysis of 16S rDNA amplified in nested polymerase chain reaction (PCR) primed by oligonucleotide pair F2n/R2 from Erigeron witches'-broom phytoplasma strain EriWB infecting a naturally diseased plant of *Erigeron* sp. First round of PCR was primed by P1/P7, followed by reamplification of target DNA in nested PCR primed by F2n/R2. DNA products from the second (nested) PCR were digested with restriction endonucleases *Alu*I, *Mse*I, *Hha*I, *Hae*III, and *Taq*I. M, Phi X174 *Hae*III digest size standard. EriWB, Erigeron witches'-broom phytoplasma strain EriWB. RPWB, Erigeron witches'-broom phytoplasma strain RPWB. AshY, "*Candidatus* Phytoplasma fraxini" strain AshY1^T. CP, clover proliferation phytoplasma.

groups, and *A. laidlawii* were aligned using Clustal X version 1.63b (34). A phylogenetic tree was constructed by the Neighbor-Joining method of the Clustal X program, and the tree was viewed by using TreeViewPPC (26). *A. laidlawii* was selected as the outgroup to root the tree. Bootstrapping was performed 1,000 times for estimation of stability and support for the clades.

RESULTS

Phytoplasma detection and RFLP analyses. The diseased plants of erigeron and periwinkle exhibited symptoms typical of phytoplasmal infections, including reduced size of leaves, chlorosis, and proliferation of axillary shoots resulting in prominent witches'-broom growths. Direct and nested PCRs primed by phytoplasma universal primer pair F2n/R2 resulted in the amplification of 1.2-kbp DNA fragments, indicating that the symptomatic erigeron and periwinkle plants were infected by phytoplasma. The phytoplasma strains detected in erigeron and periwinkle were designated Erigeron witches'-broom phytoplasma (EriWB) and Rio das Pedras witches'-broom phytoplasma (RPWB), respectively.

The detected phytoplasma strains were classified on the basis of RFLP analysis of 16S rDNA amplified in PCR primed by primer pair F2n/R2, according to the classification scheme established by Lee et al. (18,19). The collective RFLP patterns were compared with those published for other phytoplasmal 16S rDNAs (9,10,14,18,31). Strains EriWB and RPWB were indistinguishable from each other on the basis of collective RFLP patterns of rDNA from EriWB and RPWB (Fig. 1 and data not shown), consistent with the hypothesis that both plant species were infected by the same phytoplasma taxon. Based on these results, we tentatively consider EriWB and RPWB to be strains of a single phytoplasma taxon, Erigeron witches'-broom phytoplasma.

Based on the 12 RFLP profiles obtained, strains EriWB and RPWB are more closely related to groups 16SrVI (clover proliferation phytoplasma group, group VI) and 16SrVII (ash yellows phytoplasma group, group VII) than to other phytoplasma groups. EriWB and RPWB yielded *Bfa*I, *Hpa*II, *Hin*fI, *Kpn*I, *Rsa*I, *Sau*3AI, and *Tha*I RFLP patterns that were indistinguishable from those of ash yellows phytoplasma strain AshY1^T (type strain of "*Ca*. P. fraxini" and representative strain of group VII) and clover proliferation (CP) phytoplasma (representative of group VI) (data not shown). However, strains EriWB and RPWB were distinguished from CP and other previously described members of group VI by *Taq*I, *Hha*I, and *Hae*III RFLP patterns and from group VII strain AshY1^T and other phytoplasmas by *Taq*I, *Mse*I, and *Alu*I RFLP patterns of 16S rDNA (Fig. 1). The *Taq*I RFLP pattern of 16S rDNA from EriWB and RPWB appeared similar to that of rDNA from CelY phytoplasma, a member of group 16SrXII, subgroup A (stolbur phytoplasma subgroup). These results indicated that the Erigeron witches'-broom phytoplasma (strains EriWB and RPWB) was distinct from phytoplasmas described previously.

Putative restriction sites in 16S rDNA. Nucleotide sequences determined for the cloned 1.8-kbp DNA fragments from Erigeron witches'-broom phytoplasma, strains EriWB and RPWB, were deposited in the GenBank database under accession numbers AY034608 and AF411592, respectively. Expected fragment sizes based on analysis of putative restriction sites were in excellent agreement with fragment sizes obtained by enzymatic RFLP analysis of amplified DNA. Putative restriction site analysis revealed the presence of unique sites in 16S rDNA that distinguished EriWB phytoplasma from phytoplasmas previously described (11,18) as members of group 16SrVII and strains (AshY1^T, AshY3, AshY5, and LWB3) of "*Candidatus* Phytoplasma fraxini" (Fig. 2). For example, strain EriWB differed from the "*Ca*. P. fraxini" strains by the absence in EriWB 16S rDNA of an *Alu*I site that is present in the 16S rDNA of the "*Ca*. P.

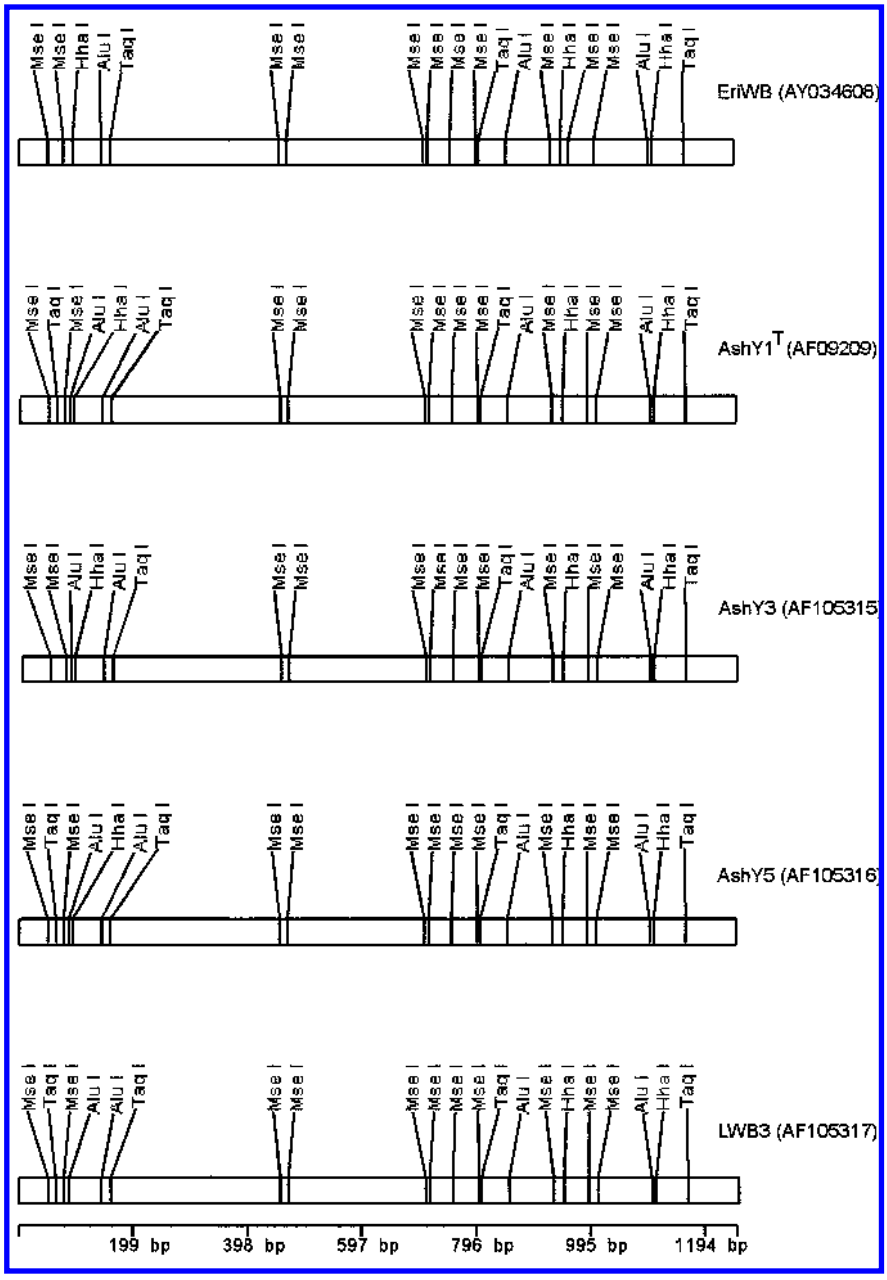

Fig. 2. Analysis of putative restriction sites in cloned 16S rDNA sequences from Erigeron witches'-broom phytoplasma strain EriWB and ash yellows phytoplasma ("*Candidatus* Phytoplasma fraxini") strains AshY1^T, AshY3, AshY5, and LWB3. GenBank numbers of the sequences are in parentheses. Each sequence represents that amplified in polymerase chain reaction (PCR) primed by oligonucleotide pair F2n/R2; phytoplasma classification into 16S rDNA groups and subgroups is based upon restriction sites in this sequence.

fraxini" strains, and by sites for *Mse*I in different positions (Fig. 2). These restriction sites that distinguished EriWB from the "*Ca*. P. fraxini" strains were located within a region that corresponds to the 1.2-kbp fragment that is amplified in PCR primed by primer pair F2n/R2 and is used for classification of phytoplasmas in the scheme of Lee et al. (18,19).

Coefficients of similarity. Coefficients of similarity in RFLP patterns of 16S rDNA were calculated using putative restriction site analysis of sequences corresponding to fragments amplified in PCRs primed by F2n/R2. The coefficients of similarity between Erigeron witches'-broom phytoplasma and two strains from group 16SrVI were 0.86 and 0.89 (Table 2), and the coefficient of similarities values between Erigeron witches'-broom phytoplasma and four strains previously classified in group 16SrVII ranged from 0.88 to 0.94 (Table 2). On the basis of these data, Erigeron witches'-broom phytoplasma was classified in group VII, new subgroup B (VII-B), because these values were within the range of coefficients of similarity reported (18) among strains within other groups.

Sequence similarity of 16S rRNA genes. Strains EriWB and RPWB shared 99.9% sequence similarity of 16S rDNA. Only one base difference at position 75, a "G" in EriWB and an "A" in RPWB 16S rDNA, distinguished the 16S rDNAs of these strains. This difference may represent normal sequence variability existing within the population of Erigeron witches'-broom phytoplasma strains. The data are consistent with the concept that EriWB and RPWB are strains of the same phytoplasma species.

Because some of the RFLP patterns of 16S rDNA from Erigeron witches'-broom phytoplasma strains are similar to those from members of groups 16SrVI and 16SrVII, further analyses were done to compare EriWB with known strains from those groups. Sequence similarities between 1.2-kbp sequences of 16S rDNA (equivalent to fragments amplified in PCRs primed by F2n/R2) from the EriWB phytoplasma and phytoplasmas affliliated with groups 16SrVI and 16SrVII were determined (Table 3). Sequence similarity with group 16SrV (elm yellows phytoplasma group) was also determined to afford comparisons with a third group.

EriWB shared 96.3% sequence similarity of 16S rDNA with EY (EY1) phytoplasma (member of group 16SrV). 16S rDNA sequence similarities between EriWB phytoplasma and two phytoplasma strains from group 16SrVI were 96.9 and 97.7%. Sequence similarities between EriWB phytoplasma and four phytoplasmas strains from group 16SrVII ranged from 98.2 to 98.5%. These data, combined with the coefficients of similarity, indicated that EriWB phytoplasma was most closely related to group VII strains among known phytoplasmas.

In classifying the Erigeron witches'-broom phytoplasma, it was desirable to reflect its relatedness to strains previously classified in group VII, and to distinguish it from the subgroup VII-A phytoplasma strains from ash and lilac in North America. The sequence similarities between 16S rDNAs of EriWB and AshY phytoplasma strains support the classification of Erigeron witches'-broom phytoplasma in group 16SrVII (ash yellows phytoplasma group) and the designation of strains EriWB and RPWB as representative of a new subgroup VII-B.

Erigeron witches'-broom phytoplasma 16S rDNA versus signature sequences of "*Ca.* P. fraxini". In the description of "*Ca.* P. fraxini", the AshY phytoplasma, two regions in the 16S rRNA gene sequence were reported as signature sequences unique to this species (11). In spite of the clear distinction, noted above, of Erigeron witches'-broom phytoplasma from "*Ca*. P. fraxini" on the basis of RFLP analysis and sequence similarity of 16S rDNA, Erigeron witches'-broom phytoplasma contains one of the two "*Ca*. P. fraxini" signature sequences and differs from the second '*Ca*. P. fraxini' signature sequence by a single base substitution. The "*Ca*. P. fraxini" signature sequence 5′-AGGAAAGTC-3′, beginning at position 588 (11), is found in Erigeron witches'-broom without base substitution. The "*Ca*. P. fraxini" signature sequence beginning at position 66 is 5′-CGGAAACCCCTCAA**A**AGGTTT-3′ (11). The corresponding sequence in EriWB phytoplasma is 5′-CGGAAACCCCTCAA**G**AGGTTT-3′.

Table 2. Similarity coefficients derived from restriction fragment length polymorphisms (RFLPs) based on putative restriction site analysis of nucleotide sequences of 16S rDNA from Erigeron witches'-broom phytoplasma and other selected phytoplasmas

Phytoplasma[a]	16S rRNA group affiliation[b]	EriWB	AshY1^T	AshY3	AshY5	LWB3	CP	MC
EriWB	VII-B	1.00						
AshY1^T	VII-A	0.91	1.00					
AshY3	VII-A	0.94	0.97	1.00				
AshY5	VII-A	0.91	1.00	0.97	1.00			
LWB3	VII-A	0.88	0.97	0.95	0.89	1.00		
CP	VI-A	0.89	0.84	0.86	0.86	0.83	1.00	
MC	VI-B	0.86	0.81	0.84	0.83	0.80	0.94	1.00

[a] EriWB, Erigeron witches'-broom phytoplasma strain EriWB. AshY1^T, AshY3, AshY5, and LWB3; strains of ash yellows phytoplasma, "*Candidatus* Phytoplasma fraxini". CP, clover proliferation phytoplasma. MC, "*Fragaria multicipita*" phytoplasma.
[b] Groups are indicated in Roman numerals. Letters indicate subgroups. Designation of phytoplasma 16S rDNA group and subgroup affiliations are according to Lee et al. (18).

Table 3. Sequence similarities among 16S rDNAs from Erigeron witches'-broom (EriWB) phytoplasma and previously described phytoplasma strains belonging to groups 16SrVI and 16SrVII

Phytoplasma[a]	16S rDNA group-subgroup affiliation[b]	EriWB	AshY1^T	AshY3	AshY5	LWB3	CP	MC
EriWB	VII-B	100						
AshY1^T	VII-A	98.5	100					
AshY3	VII-A	98.5	99.8	100				
AshY5	VII-A	98.5	100	99.8	100			
LWB3	VII-A	98.2	99.7	99.5	99.7	100		
CP	VI-A	97.7	97.0	97.0	97.0	96.7	100	
MC	VI-B	96.9	96.6	96.6	96.6	96.3	99.0	100

[a] EriWB, Erigeron witches'-broom phytoplasma strain EriWB. AshY1^T, AshY3, AshY5, and LWB3; strains of ash yellows phytoplasma, "*Candidatus* Phytoplasma fraxini". CP, clover proliferation phytoplasma. MC, "*Fragaria multicipita*" phytoplasma.
[b] Roman numerals indicate 16S rDNA groups. Subgroups are indicated by letters. Designation of group and subgroup affiliations are according to Lee et al. (18).

These findings emphasize the close relationship of EriWB phytoplasma to ash yellows and lilac witches'-broom phytoplasmas classified in group VII.

Phylogenetic analysis. Phylogenetic analysis of 16S rRNA gene sequences from 26 phytoplasma strains and *A. laidlawii* yielded a tree whose branching order is in general agreement with previous findings (5,11,18,19) (Fig. 3). Erigeron witches'-broom phytoplasma emerges as a new branch, not seen in previous phylogenetic trees. The branching order indicates that ash yellows, lilac witches'-broom, and Erigeron witches'-broom phytoplasmas descended from a common ancestor. The branching also indicates that EriWB phytoplasma represents a new lineage, supporting the distinction of this phytoplasma from ash yellows phytoplasma strains previously described as strains of "*Ca*. P. fraxini".

DISCUSSION

In this communication, we describe the discovery in Brazil of a new member of the ash yellows phytoplasma group, group 16SrVII. We have placed the Erigeron witches'-broom phytoplasma in a new subgroup (VII-B) of group 16SrVII, indi-

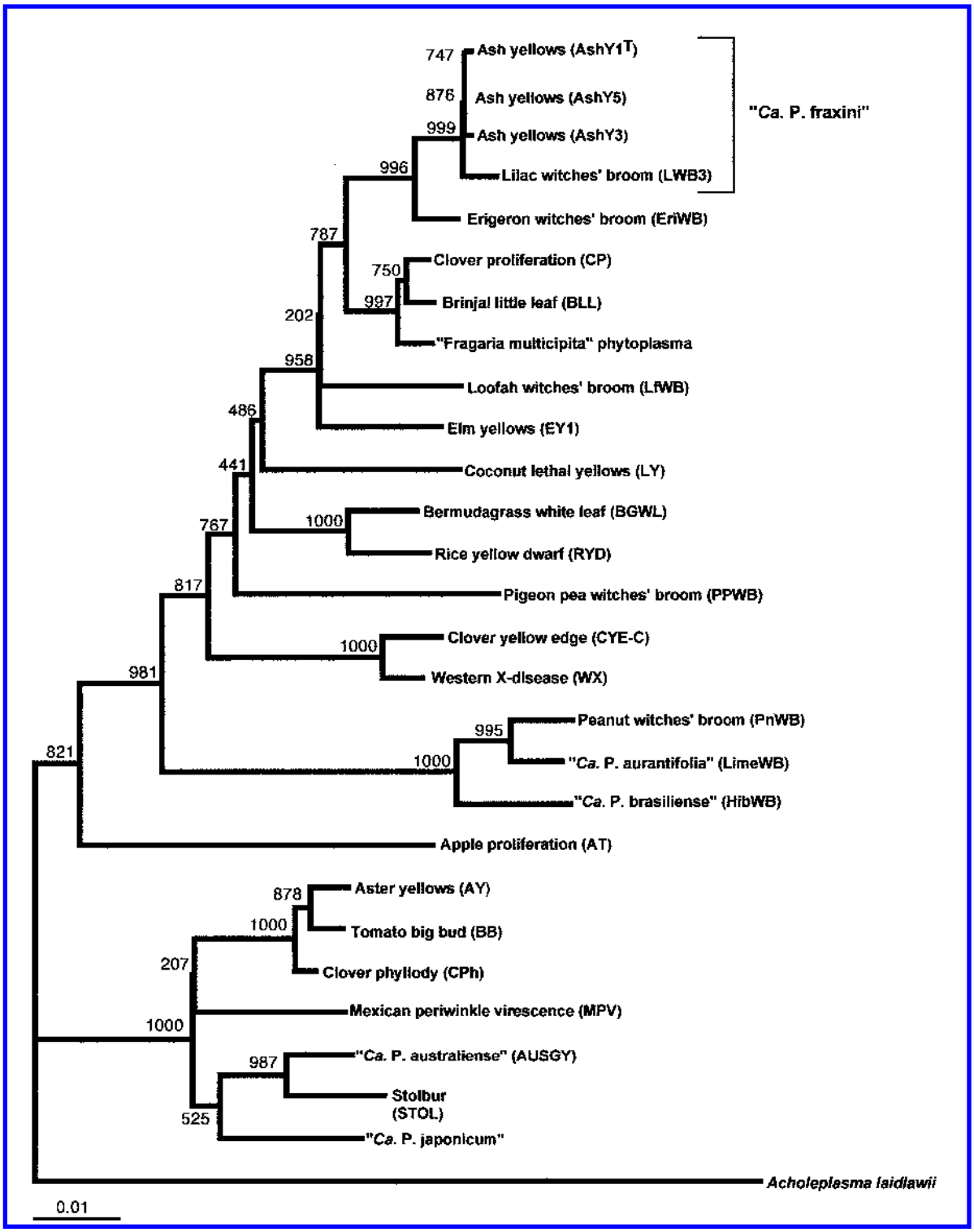

Fig. 3. Phylogenetic tree constructed by the Neighbor-Joining method of 16S rRNA gene sequences from 27 phytoplasmas and *Acholeplasma laidlawii*, employing *A. laidlawii* as the outgroup. The numbers on the branches are bootstrap (confidence) values. Phytoplasma strain designations are in parentheses.

cating that it possesses unique properties that are reflected in the results from enzymatic RFLP analyses, sequence similarities, coefficients of similarity, and phylogenetic analysis based on 16S rRNA gene sequences. Phytoplasmas previously described in Brazil belong to four distinct phylogenetic groups: the aster yellows phytoplasma group (16SrI) (2,7), the X-disease phytoplasma group (16SrIII) (1,7,23), the pigeon pea witches'-broom phytoplasma group (16SrIX) (3), and the hibiscus witches'-broom group (16SrXV) (22). Erigeron witches'-broom phytoplasma is the first of the ash yellows phytoplasma group (group 16SrVII) to be reported in Brazil.

The present study and other work recently published in abstract form are the first to report members of the ash yellows phytoplasma group outside of North America (8,21; this paper). Previously, ash yellows has been a disease known only on the North American continent, where it is apparently restricted to species of *Syringa* and *Fraxinus*, members of the family Oleaceae (11,31,32). These two genera do not occur naturally in Brazil, but *Fraxinus* is cultivated as an ornamental in some regions. Other genera in the Oleaceae, including *Linociera* and *Chionantus*, are native to South America, and it would be of interest to learn whether these latter genera may be infected by Erigeron witches'-broom or other phytoplasmas affiliated with group 16SrVII (group VII). Griffiths et al. (8) reported ash yellows disease in *Fraxinus chinensis* in Columbia, and Meneguzzi et al. (21) reported a group VII phytoplasma in diseased alfalfa in Argentina, but nucleotide sequences of the phytoplasmas' 16S rRNA genes were not reported. It would be interesting to know the relationships among group VII phytoplasmas in Columbia (8) and Argentina (21), ash yellows phytoplasma strains in North America (11), and Erigeron witches'-broom phytoplasma in Brazil (this study).

More than 200 species of *Erigeron* are known on the North American continent, where they are widespread. Although *Erigeron* sp. is not native to Brazil, the genus now is widespread and adapted to local ecological conditions in Brazil. Species of *Erigeron* were first described as hosts of phytoplasmas in Brazil by Kitajima and Costa (15,16), on the basis of phytoplasma-characteristic disease symptoms in plants and the observation of phytoplasma cells in phloem of diseased plants examined by electron microscopy. In addition to group VII phytoplasma strains reported in the present study, an undescribed phytoplasma belonging to group 16SrI (aster yellows phytoplasma group) has been reported in plants of *Erigeron bonariensis* with symptoms of witches'-broom (4). Thus, it is apparent that species of *Erigeron* may serve as hosts for divergent phytoplasma lineages in Brazil. The presence of Erigeron witches'-broom phytoplasma strains in both *Erigeron* sp. and periwinkle plants, as shown in the present study, suggests that at least one polyphagous insect is capable of transmitting this phytoplasma in Brazil. This raises the possibility that other, as yet undiscovered, plant hosts of group VII phytoplasma strains exist in South America.

To date, 15 major groups and over 34 subgroups of phytoplasmas have been reported. Each major group represents at least one species (22); the appropriate taxonomic rank of many recognized subgroups is not yet clear (18). Because it is not possible to obtain phytoplasmas in pure culture in vitro, a provisional system of naming distinct phytoplasmas as "*Candidatus* Phytoplasma species" has been adopted, and several such species have been distinguished and described principally on the basis of results from analysis of 16S rRNA gene sequences (6,11,22,28,35,36). For example, group 16SrVII (ash yellows phytoplasma group) was designated "*Candidatus* Phytoplasma fraxini" (11). Although Erigeron witches'-broom phytoplasma is a member of group 16SrVII, data in the present study clearly indicate that it represents a subgroup distinct from strains presently encompassed in this species. Conceivably, ecological and/or geographical isolation could have fostered evolutionary divergence of Erigeron witches'-broom phytoplasma from its closest known relatives. Yet it is not clear that this distinction is of species level character, particularly because Erigeron witches'-broom phytoplasma and "*Ca*. P. fraxini" strains share a 16S rDNA sequence similarity that is greater than the current species level threshold of 97.5%, and Erigeron witches'-broom phytoplasma contains 16S rDNA signature sequences reported for "*Ca*. P. fraxini" (this paper). Further work is needed to assess the taxonomic significance of 16S rRNA gene sequence divergence and other properties of Erigeron witches'-broom phytoplasma in order to determine whether it represents a distinct "*Candidatus* Phytoplasma species".

ACKNOWLEDGMENTS

We thank Jim Plaskowitz for help in preparing figures in this manuscript and Rasa Jomantiene for helpful suggestions during the writing of the manuscript.

LITERATURE CITED

1. Barros, T. S. L., Kitajima, E. W., and Resende, R. O. 1998. Divergence of Brasilian phytoplasmas based on analyses of 16S rDNA. Fitopatol. Bras. 23:459-465.
2. Bedendo, I. P., Davis, R. E., and Dally, E. L. 1997. Molecular evidence for the presence of maize bushy stunt phytoplasma in corn in Brazil. Plant Dis. 81:957.
3. Bedendo, I. P., Davis, R. E., and Dally, E. L. 1999. Detecção e caracterização de fitoplasmas em plantas de vinca (*Catharanthus roseus*) e de pimenta (*Capsicum frustescens*) através das técnicas de duplo PCR e de RFLP. Summa Phytopathol. 25:197-201.
4. Bianchini, L., and Bedendo, I. P. 2000. Presença de um fitoplasma do grupo I, evidenciado por PCR, em plantas de Erigeron (*Erigeron bonariensis*) com superbrotamento. (Abstr.) Fitopatol. Bras. 25:348.
5. Davis, R. E., and Dally, E. L. 2001. Revised subgroup classification of group 16SrV phytoplasmas and placement of *flavescence dorée*-associated phytoplasmas in two distinct subgroups. Plant Dis. 85:790-797.
6. Davis, R. E., Dally, E. L., Gundersen, D. E., Lee, I.-M., and Habili, N. 1997. "*Candidatus* Phytoplasma australiense," a new phytoplasma taxon associated with australian grapevine yellows. Int. J. Syst. Bacteriol. 47:262-269.
7. Davis, R. E., Dally, E. L., Lee, I.-M., Resende, R. O., and Kitajima, E. W. 1994. Detection and characterization of mycoplasmalike organisms (MLOs) in diverse plant species in Brazil. (Abstr.) Fitopatol. Bras. 19:340.
8. Griffiths, H. M., Boa, E. R., and Filgueira, J. J. 2001. Ash yellows: A new disease of *Fraxinus chinensis* in Columbia. (Abstr.) Phytopathology 91:S32-33.
9. Griffiths, H. M., Sinclair, W. A., Boudon-Padieu, E., Daire, X., Lee, I.-M., Sfalanga, A., and Bertaccini, A. 1999. Phytoplasmas associated with elm yellows: Molecular variability and differentiation from related organisms. Plant Dis. 83:1101-1104.
10. Griffiths, H. M., Sinclair, W. A., Davis, R. E., Lee, I.-M., Dally, E. L., Guo, Y.-H., Chen, T. A., and Hibben, C. R. 1994. Characterization of mycoplasmalike organisms from *Fraxinus*, *Syringa,* and associated plants from geographically diverse sites. Phytopathology 84:119-126.
11. Griffiths, H. M., Sinclair, W. A., Smart, C. D., and Davis, R. E. 1999. The phytoplasma associated with ash yellows and lilac witches'-broom: '*Candidatus* Phytoplasma fraxini'. Int. J. Syst. Bacteriol. 49:1605-1614.
12. Gundersen, D. E., and Lee, I.-M. 1996. Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. Phytopathol. Mediterr. 35:144-151.
13. Gundersen, D. E., Lee, I.-M., Rehner, S. A., Davis, R. E., and Kingsbury, D. T. 1994. Phylogeny of mycoplasmalike organisms (phytoplasmas): A basis for their classification. J. Bacteriol. 176:5244-5254.
14. Jomantiene, R., Davis, R. E., Maas, J., and Dally, E. L. 1998. Classification of new phytoplasmas associated with diseases of strawberry in Florida, based on analysis of 16S rRNA and ribosomal protein gene operon sequences. Int. J. Syst. Bacteriol. 48:269-277.
15. Kitajima, E. W., and Costa, A. S. 1970. Micoplasma, possível agente etiológico de certas moléstias de plantas. Ciência Cultura 22:351-363.
16. Kitajima, E. W., and Costa, A. S. 1979. Microorganismos do tipo micoplasma associados a moléstias do tipo amarelo em algumas plantas cultivadas e ornamentais, no estado de São Paulo e no Distrito Federal. Fitopatol. Bras. 4:317-327.
17. Lee, I.-M., Davis, R. E., and Gundersen-Rindal, D. E. 2000. Phytoplasma: Phytopathogenic mollicutes. Annu. Rev. Microbiol. 54:221-255.
18. Lee, I.-M., Gundersen-Rindal, D. E., Davis, R. E., and Bartoszyk, I. M. 1998. Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. Int. J. Syst. Bacteriol. 48:1153-1169.
19. Lee, I.-M., Hammond, R. W., Davis, R. E., and Gundersen, D. E. 1993. Universal ampli-

fication and analysis of pathogen 16S rDNA for classification and identification of mycoplasmalike organisms. Phytopathology 83:834-842.
20. Lim, P.-O., and Sears, B. B. 1989. 16S rRNA sequence indicates that plant-pathogenic mycoplasmalike organisms are evolutionarily distinct from animal mycoplasmas. J. Bacteriol. 171:5901-5906.
21. Meneguzzi, N., Torres, L., Galdeano, E., Nome, C., Docampo, D., Nome, S., and Conci, L. 2001. Phylogenetic analysis of an ash yellows group phytoplasma detected in alfalfa (*Medicago sativa* L.), in Argentina. (Abstr.) Fitopatol. Bras. 26(Supl.):510.
22. Montano, H. G., Davis, R. E., Dally, E. L., Hogenhout, S., Pimentel, J. P., and Brioso, P. S. T. 2001. '*Candidatus* Phytoplasma brasiliense', a new phytoplasma taxon associated with hibiscus witches' broom disease. Int. J. Syst. Evol. Microbiol. 51:1109-1118.
23. Montano, H. G., Davis, R. E., Dally, E. L., Pimentel, J. P., and Brioso, P. S. T. 2000. Identification and phylogenetic analysis of a new phytoplasma from diseased chayote in Brazil. Plant Dis. 84:429-436.
24. Namba, S., Oyaizu, H., Kato, S., Iwanami, S., and Tsuchizaki, T. 1993. Phylogenetic diversity of phytopathogenic mycoplasma-like organisms. Int. J. Syst. Bacteriol. 43:461-467.
25. Nei, M., and Li, W.-H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76:5269-5273.
26. Page, R. D. 1996. TreeView: An application to display phylogenetic trees on personal computers. Comput. Appl. Biosci. 12:357-358.
27. Prince, J. P., Davis, R. E., Wolf, T. K., Lee, I.-M., Mogen, B. D., Dally, E. L., Bertaccini, A., Credi, R., and Barba, M. 1993. Molecular detection of diverse mycoplasmalike organisms (MLOs) associated with grapevine yellows and their classification with aster yellows, X-disease, and elm yellows MLOs. Phytopathology 83:1130-1137.
28. Sawayanagi, T., Horikoshi, N., Kanehira, T., Shinohara, M., Bertaccini, A., Cousin, M. T., Hiruki, C., and Namba, S. 1999. '*Candidatus* Phytoplasma japonicum', a new phytoplasma taxon associated with Japanese hydrangea phyllody. Int. J. Syst. Bacteriol. 49:1275-1285.
29. Schneider, B., Cousins, M. T., Klinkong, S., and Seemüller, E. 1995. Taxonomic relatedness and phylogenetic position of phytoplasmas associated with diseases of faba bean, sunnhemp, sesame, soybean, and eggplant. Z. Pflanzenkrankh. Pflanzenschutz 102:225-232.
30. Seemüller, E., Schneider, B., Mäurer, R., Ahrens, U., Daire, X., Kison, H., Lorenz, K. H., Firrao, G., Avinent, L., and Sears, B. B. 1994. Phylogenetic classification of phytopathogenic mollicutes by sequence analysis of 16S ribosomal DNA. Int. J. Syst. Bacteriol. 44:440-446.
31. Sinclair, W. A., and Griffiths, H. M. 2000. Variation in aggressiveness of ash yellows phytoplasmas. Plant Dis. 84:282-288.
32. Sinclair, W. A., Griffiths, H. M., and Davis, R. E. 1996. Ash yellows and lilac witches'-broom: Phytoplasmal diseases of concern in forestry and horticulture. Plant Dis. 80:468-475.
33. Smart, C. D., Schneider, B., Blomquist, C. L., Guerra, L. J., Harrison, N. A., Ahrens, U., Lorenz, K.-H., Seemüller, E., and Kirkpatrick, B. C. 1996. Phytoplasma-specific PCR primers based on sequences of the 16S-23S spacer region. Appl. Environ. Microbiol. 62:2988-2993.
34. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. 1997. The Clustal X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25:4876-4882.
35. White, D. T., Blackall, L. L., Scott, P. T., and Walsh, K. B. 1998. Phylogenetic positions of phytoplasmas associated with dieback, yellow crinkle and mosaic diseases of papaya, and their proposed inclusion in '*Candidatus* Phytoplasma australiense' and a new taxon, '*Candidatus* Phytoplasma australasia'. Int. J. Syst. Bacteriol. 48:941-951.
36. Zreik, L., Carle, P., Bové, J. M., and Garnier, M. 1995. Characterization of the mycoplasmalike organism associated with witches'-broom disease of lime and proposition of a *Candidatus* taxon for the organism, "*Candidatus* Phytoplasma aurantifolia". Int. J. Syst. Bacteriol. 45:449-453.