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Phytoplasmas diversity and identification of new aster yellows subgroup (16SrI) associated with weed species in Argentina

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Abstract

Symptoms of phytoplasma infection were observed in different weed species, *Bidens subalternans*, *Conyza bonariensis*, *Heterosperma ovatifolium* and *Conium maculatum*, collected from diverse geographical regions in Argentina. To confirm the association of phytoplasma infection with symptomatic plants, PCR, RFLP and phylogenetic analyses based on 16S rRNA-encoding sequences were performed. In this work, we report the presence of phytoplasmas from group 16SrVII (subgroup 16VII-B) infecting *C. bonariensis* and *B. subalternans* and from group 16SrIII (subgroup 16SrIII-X) *B. subalternans*, *H. ovatifolium*, and *C. maculatum*. Phytoplasmas from the aster yellows group were detected infecting *C. bonariensis* and *B. subalternans*. Analysis of 16S rRNA-encoding genes revealed the presence of two distinct operons, *rrnB* (16SrI-B) and newly described *rrnA*, which is different from the reference RFLP patterns of all previously established 16SrI-subgroups. A single *rp* operon sequence analysis reveals the presence of simple infection and confirms a description of a novel subgroup. On the basis of these results we propose a designation of new subgroup 16SrI-(B/AJ) AJ (*rp*-AJ). To our knowledge, this is the first report of phytoplasmas infecting *Bidens subalternans* , *Heterosperma ovatifolium* and *Conium maculatum*.

Phytoplasmas are cell-wall-less bacteria, with small AT-rich genomes encoding capabilities for a transkingdom parasitic lifestyle, on plants and insects [1]. These pathogens are associated with diseases in more than 1000 plant species worldwide, including crops, ornamentals and weeds [2]. In nature the plant-to-plant transmission is caused by the action of phloem-feeding insects of the order Hemiptera, primarily leafhoppers, planthoppers and psyllids [3]. In spite of numerous efforts, isolation and axenic cultivation of phytoplasmas remains a major challenge, complicating the development of diversity studies [4]. However, genotypic approaches have been useful for the classification and taxonomy of phytoplasmas. On the basis of 16S rRNA gene sequence and RFLP profile analyses, a comprehensive classification scheme has been delineated to classify phytoplasmas into groups and subgroups [5]. Nowadays, based on the information provided by this system and using a virtual RFLP program [6], 35 16 S rRNA groups and more than 100 subgroups have been determined [7].

In Argentina, diversity studies allowed the identification of five 16 Sr phytoplasma groups (16SrI, 16SrIII, 16SrVII, 16SrX and 16SrXIII) associated with several plant hosts [8, 9]. Most of the phytoplasmas that have been detected are unique to South America and have not been found on other continents [10–13]. Many of them have been found infecting weeds or endemic plant species [11, 14] and also in naturally infected insects [15–19], which could play a role as reservoirs and vectors respectively. The identification of weeds that can act as phytoplasmas' natural reservoirs is of vital importance both for understanding the pathosystems and for the management of diseases.

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Keywords: Phytoplasma diversity; weeds; phyllody; witches' broom; classification.
Abbreviations: RFLP, Restriction Fragment Length Polymorphism; rRNA, ribosomal RNA; rrnA, rrnB, operon heterogeneity; SNP, Single Nucleotide Polymorphism.
The sequences studied have been deposited in GenBank/EMBL/DDBJ with the following accession numbers: BidPhy-Mis2015 16S rRNA gene sequence (MH497012), ConWBr-Mza2015 16S rRNA gene sequence (MH497015), BidPhy-SJQ2016 16S rRNA gene sequence (MH497013), ConPhy-SJQ2015 16S rRNA gene sequence (MH497017), HetPhy-SJQ2015 16S rRNA gene sequence (MH497018), BidPhy-Cba2017 16S rRNA gene sequence (MH497011) *rrnA*, ConWBr-Sgo2016 (MH4977016) *rrnA*, ConWBr-Ju2017 (MH4977014) *rrnA*, BidPhy-Cba2017 (MK881081) *rrnB*, ConWBr-Sgo2016 (MK881082) *rrnB*, ConWBr-Ju2017 (MK881080) *rrnB*; and rp-sequences BidPhy-Cba2017 (MK896922), ConWBr-Ju2017 (MK896923) and ConWBr-Sgo2016 (MK896924).
Seven supplementary figures and four supplementary tables are available with the online version of this article.

Between 2015 and 2017, different weed species with phytoplasma infection symptoms were collected from different regions of Argentina (Fig. S1, available in the online version of this article). Samples with phytoplasma symptomatology (phyllody or witches' broom) were collected from 'hairy fleabane' [*Conyza bonariensis* (L.) Cronquist]; 'beggar-ticks' (*Bidens subalternans*); *Heterosperma ovatifolium*; and 'hemlock' (*Conium maculatum* M.) (Table 1, Fig. 1). Asymptomatic samples of each species were also collected and used as negative controls. Total nucleic acid was purified using the Doyle & Doyle protocol [20]. Phytoplasma detection was acceded by PCR using the universal primer pairs P1–P7 (1.8 kb) [21] and R16F2n–R16R2 (1.2 kb) [22], in direct and nested reactions as previously stated [11]. Analysis of RFLP patterns from PCR-positive samples (1.2 kb) were conducted using the enzymes *Mse*I, *Hha*I, *Rsa*I, *Hinf*I and *Taq*I (NEB) according to the manufacturer's instructions. The RFLP profiles were resolved in agarose: MetaPhor (1.5×:0.5×) electrophoresis gels, stained with GelRed and visualized with a UV transilluminator. Restriction patterns were screened in order to detect the presence of supernumerary bands as an indication of operon heterogenicity. In molecular analyzes, one representative phytoplasma sample from each host/location was selected and the 16S rRNA-encoding gene partial sequence was obtained. PCR amplifications (primers R16F2n–R16R2) were cloned in pGEM-T Easy Vector Systems (Promega) and transformed into *Escherichia coli* DH5α competent cells. Three to six different clones were sequenced from both extremes in an automatic sequencer service (Unidad de Genómica, Instituto de Biotecnología-CICyVA; INTA, Buenos Aires, Argentina). Final consensus sequences (minimum 3× coverage) were assembled using the Geneious R10 software and were deposited in a public database (NCBI). Assignment of 16 Sr group/subgroup was obtained by analyzing the global *in silico* RFLP-pattern using the *i*PhyClassifier program [6]. Also, phylogenetic relationships were inferred by the maximum likelihood method using the MEGA6 software [23]. Sequences for phylogenetic analyses were selected on the basis of the classification provided by the virtual RFLP profiles.

IDENTIFICATION OF PHYTOPLASMAS FROM ASH YELLOWS GROUP (16SRVII)

Samples of *Conyza bonariensis* and *Bidens subalternans* with witches' broom and phyllody symptoms, respectively, were positive for phytoplasmas in PCR reactions (Table 1). No amplification was observed in asymptomatic samples. The actual PCR–RFLP profiles obtained with *HhaI*, *Hinf*I and *Taq*I were identical among all the samples and indicated that the novel strains were related to the 16SrVII group (Fig. S3). The presence of supernumerary bands was not detected. Partial sequences of 16S rRNA-encoding genes were obtained from two representative samples, one from *Conyza bonariensis* (ConWBr-Mza2015; MH497015) and one from *B. subalternans* (BidPhy-Mis2015; MH497012). The global virtual RFLP profile indicated that both samples are variants of the 16SrVII-B subgroup (similarity coefficient=0.99) (Table S1 and Fig. S1). Regarding the phylogenetic relationships, the final tree showed a topology (Fig. 2) similar to those described in previous work [24, 25]. The ConWBr-Mza2015 and BidPhy-Mis2015 samples were grouped in the same clade within representative sequences from subgroups 16SrVII-B, -D and -F. At present, six subgroups (A, B, C, D, E and F) have been described within the ash yellows (16SrVII) [25]. The 16SrVII-B subgroup was only recorded in South America in association to several plant species including herbaceous, such as *Erigeron* sp., periwinkle [26], *Conyza bonariensis*, *Artemisia annua* [14]; cultivated cauliflower [27]; and the evergreen shrub *Polyscias fruticosa* [28]. In this work we extend the host range of subgroup 16SrVII-B by including *B. subalternans* as novel host. *B. subalternans* is an annual herb original to South America, frequently considered a weed of summer crops [29]. Interestingly, subgroups 16SrVII-B, -C, D- and -F are associated with herbaceous species [24, 25, 30] while 16SrVII-A and -E are more likely to be in association with woody species [31, 32]. Our findings follow this pattern, adding a herbaceous species to the host repertory of 16SrVII-B subgroup.

IDENTIFICATION OF PHYTOPLASMAS FROM X-DISEASE (16SRIII)

Samples from *Bidens subalternans, Heterosperma ovatifolium*, and *Conium maculatum* displaying symptoms of phyllody and witches' broom were collected from roadsides and fields in Córdoba (Table 1, Fig. S1). Phytoplasma infections were confirmed by PCR in all symptomatic samples. No amplification was observed in asymptomatic samples. Actual RFLP patterns obtained with enzymes *Hae*III, *Mse*I, *Hinf*I and *Rsa*I were identical among all samples and indicated that they were related to the X-disease group (Fig. S5). For these samples, the presence of supernumerary bands in the RFLP profiles was not recorded either. Three representative samples were sequenced; BidPhy-SJQ2016 (MH497013); HetPhy-SJQ2015 (MH497018) and ConPhy-SJQ2015 (MH497017) and virtual RFLP profiles were analyzed. Based on *i*PhyClassifier results, phytoplasmas BidPhy-SJQ2016 ConPhy-SJQ2015 and HetPhy-SJQ2015 were grouped within the 16SrIII-X subgroup (similarity coefficient=1). In the phylogenetic tree, the samples obtained in this work were clustered within a major clade that comprised the representative strain of 16SrIII-X and three novel 16SrIII-undenominated subgroups associated with Strawberry X redness disease [33]. The X-disease is the most important and widely distributed phytoplasma group in South America, with several plant species affected [11, 13]. The 16SrIII-X subgroup was only recorded in association with witches' broom in *Conyza bonariensis* plants collected from Córdoba. *Conium maculatum* or 'cicuta' is a biennial herb native from Europe and naturalized in Argentina and Uruguay, which grows frequently in low and humid places where it became a weed. *Heterosperma ovatifolium* is an annual herb native to South America, which is frequently found as a weed in orchards, parks and gardens, especially in shaded areas [29]. Here we report for the first time, to our knowledge, the presence of phytoplasmas form subgroup

Table 1. Detection and identification of phytoplasmas on weeds collected from different locations in Argentina

Phy: Phyllody, WBr: Witches' broom.

Host	Symptom	Province,year	Number of samples	PCR (+)*	Phytoplasma (acronym)	16Sr†	Accession number
Bidens subalternans	Phy–WBr	Córdoba,2016	6	6	Bidens Phyllody (BidPhy-SJQ)	16SrIII-X	MH497013
	Phy–WBr	Córdoba,2017	3	3	Bidens Phyllody (BidPhy-Cba)	16SrI-AJ‡	MH497011
	Phy–WBr	Misiones,2015	3	3	Bidens Phyllody (BidPhy-Mis)	16SrVII-B	MH497012
Heterosperma ovatifolium	Phy–WBr	Córdoba,2015	3	3	Heterosperma Phyllody (HetPhy-SJQ)	16SrIII-X	MH497018
Conium maculatum L.	Phy–WBr	Córdoba,2015	5	5	Conium Phyllody (ConPhy-SJQ)	16SrIII-X	MH497017
Conyza bonariensis (L.) Cronquist	WBr	Mendoza,2015	3	3	Conyza Witches' broom (ConWBr-Mza)	16SrVII-B	MH497015
	WBr	Buenos Aires,2017	2	2	Conyza Witches' broom (ConWBr-Ju)	16SrI-AJ‡	MH497014
	WBr	Santiago del Estero,2016	3	3	Conyza Witches' broom (ConWBr-Sgo)	16SrI-AJ‡	MH497016

*Samples positive for PCR reactions using universal primers set P1P7 and R16F2/R16R2
†16Sr-subgroup assigned by analysis of 16S rRNA partial sequence (1241 bp) using *i*PhyClassifier
‡novel subgroup described in this work.

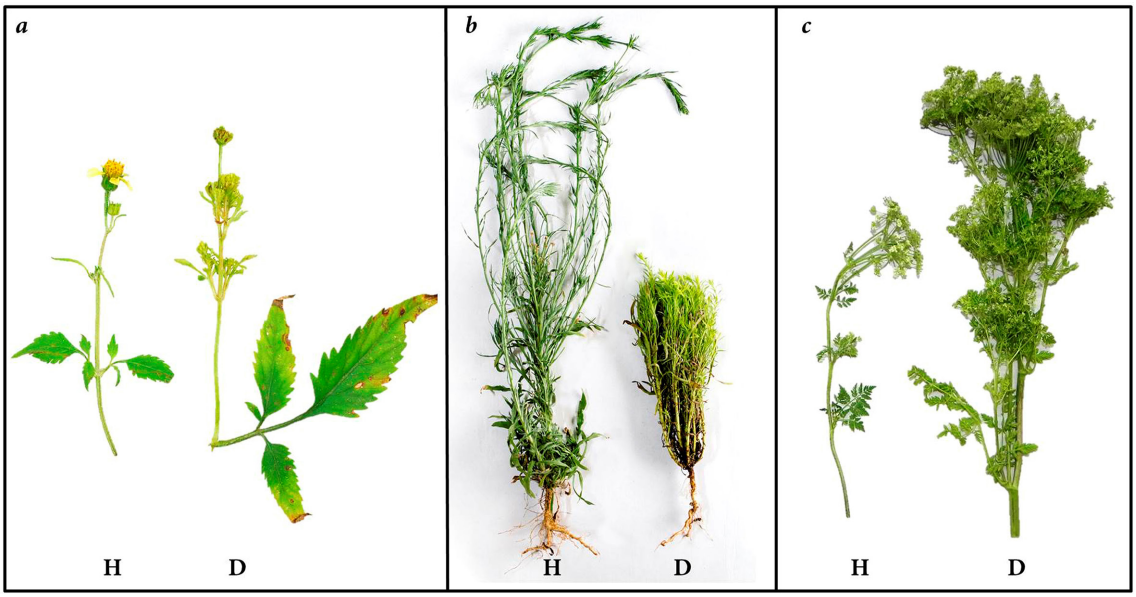

Fig. 1. Symptomatology in weeds infected with phytoplasmas. (a) *Bidens subalternans* showing phyllody; (b) *Conyza bonariensis* showing witches' broom and (c) *Conium maculatum* showing symptoms of witches' broom and phyllody. H: healthy plant, D, diseased plant.

16SrIII-X affecting *Bidens subalternans*, *Conium maculatum* and *Heterosperma ovatifolium*. Since infections of subgroup 16SrIII-X phytoplasma have been reported in closely neighboring areas [11], we presume that one or more insect vectors exist that could be disseminating the disease among regional herbaceous species.

NEW PHYTOPLASMA SUBGROUP 16SRI-(B/AJ) AJ, *RP*-AJ ASSOCIATED WITH PHYLLODY IN *CONYZA BONARIENSIS* AND *BIDENS SUBALTERNANS*

Samples with witches' broom and witches' broom and phyllody from *Conyza bonariensis* and *Bidens subalternans* plants, respectively, were collected in different geographical regions (Table 1, Fig. S1). Phytoplasma infections were detected by PCR in all symptomatic samples while no amplification was observed in asymptomatic ones. Actual RFLP profiles of *Rsa*I and *Hae*III enzymes were indistinguishable from those of the Argentinean Catharanthus Litle Leaf phytoplasma (ACLL), used as a reference strain for the aster yellows group (16SrI) (Fig. S7). As previously reported for the ACLL phytoplasma [34], the patterns revealed the presence of supernumerary bands, indicating 16S rRNA-operon heterogeneity. Partial 16S rDNA gene operon sequences of ConWBr-Ju2017 (*rrnA* MH497014, *rrnB* MK881080), ConWBr-Sgo2016 (*rrnA* MH497016, *rrnB* MK881082) and BidPhy-Cba2017 (*rrnA* MH49701, *rrnB* MK881081) phytoplasmas were analyzed. The virtual RFLP analyses performed with the *i*PhyClassifier program indicated that *rrnB* sequences of ConWBr-Ju2017, ConWBr-Sgo2016 and BidPhy-Cba2017 had RFLP profiles identical (F=1) with those of members of subgroup 16SrI-B (reference sequence: AP006628) (Table S3). The *rrnA* sequences, instead, had an identical collective profile (Table S3), but different from the reference patterns of previously established 16 Sr groups/subgroups. The most similar was the reference pattern of subgroup 16SrI-Z (GenBank accession number AY725209), with a similarity coefficient of 0.95. The key enzymes that distinguished the *rrnA* pattern from 16SrI-Z subgroup were *Rsa*I and *Dra*I (Fig. 3). The phylogenetic analyses supported these results, since the *rrnA* operon sequences (Fig. 2) were grouped in the same branch with the ACLL *rrnA* operon. To rule out the presence of mixed infections with related phytoplasmas, we analyzed the ribosomal protein genes sequences, which are present as single copies, and are also commonly used for finer differentiation in classification of phytoplasmas [35]. When the *rp*-operon sequences obtained in this work (MK86922–MK86924) were compared with those representatives of different 16SrI-subgroups, six SNPs were detected (Table S4). These differences were consistent with the phylogenetic analyses since the sequences described here clustered together (boot=99) in a clade separated from subgroups *rp*I-D and *rp*I-B (Fig. 4). *In silico* rp-RFLP profile analysis indicated that the enzymes *Tsp*509I and *Mse*I could distinguish the strain described in this work from subgroups *rp*I-D and *rp*I-B profiles (Fig. 5). On the basis of these results, we propose the designation of a novel subgroup 16SrI-(B/AJ) AJ associated with phyllody and witches' broom in *B. subalternans* and *C. bonariensis*, respectively.

The analyses of 16 s rDNA and ribosomal protein genes indicated that ConWBr-Ju2017, ConWBr-Sgo2016 and BidPhy-Cba2017 phytoplasmas are identical with ACLL phytoplasma previously detected infecting *Catharanthus roseus* in

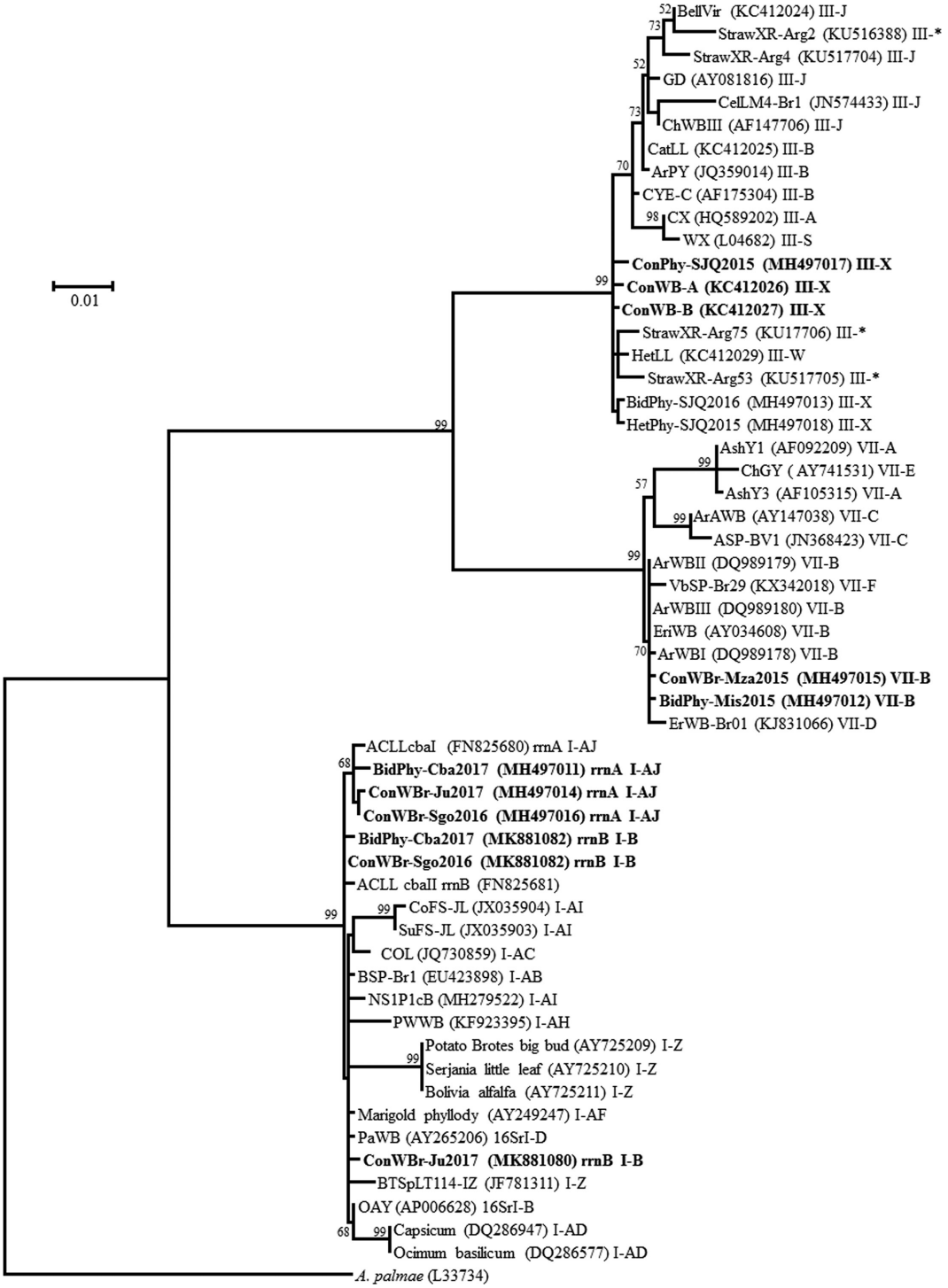

Fig. 2. Phylogenetic relationships among phytoplasmas detected in weeds from Argentina and representative sequences from aster, ash and X-disease groups. The evolutionary history was inferred from analysis of 16S rRNA gene sequences using the maximum-likelihood method implemented in MEGA 6 [23]. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. *Acholeplasma palmae* was used as outgroup. Sequences obtained in this work are in bold type. The corresponding 16Sr-subgroup was added to each taxon. *, Novel 16SrIII subgroup with no assigned letter [33].

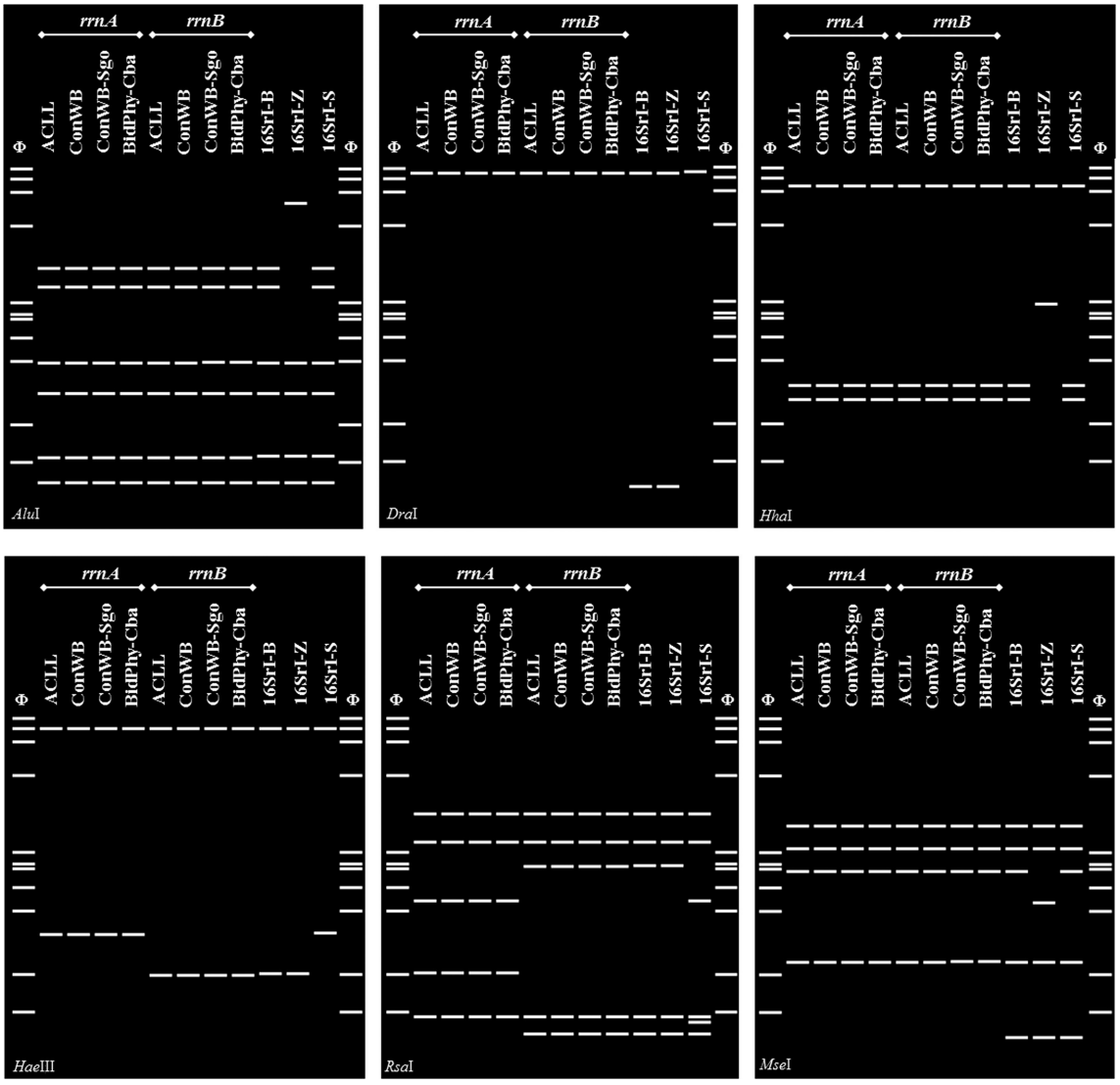

Fig. 3. RFLP patterns derived from *in silico* RFLP analysis, using *i*PhyClassifier, of 16S rDNA R16F2n/R2 fragments from 16SrI-B (AP006628), 16SrI-Z (AY725209) and 16SrI-S (AF222066) subgroups references sequences. *rrn*A operon sequences: ACLL (FN825680), BidPhy-Cba2017 (MH497011), ConWBr-Sgo2016 (MH4977016) and ConWBr-Ju2017 (MH4977014). *rrn*B operon sequences: ACLL (FN825681), BidPhy-Cba2017 (MK881081), ConWBr-Sgo2016 (MK881082) and ConWBr-Ju2017 (MK881080). Enzymes: *Alu*I, *Dra*I, *Hha*I, *Hae*III, *Rsa*I and *Mse*I; φ: ϕ X174-HaeIII digest.

Argentina and described as a member of subgroup 16SrI-S [34]. At the same time, Lilac Little Leaf (LcLL) phytoplasma was also reported as representing 16SrI-S subgroup [36], and since then has been generally accepted as 16SrI-S subgroup in further classifications. For such reasons we propose the reassignment of ACLL phytoplasma into the novel subgroup 16SrI-(B/AJ) AJ.

The aster yellows group is the largest and most diverse phytoplasma group distributed worldwide [1]. In South America, phytoplasmas from this group have been found affecting diverse species as alfalfa, potato, mora-mora vine, sugarcane, grapevine, corn, *Fraxinus uhdei* and *Populus nigra* [13]. In Argentina, phytoplasmas from subgroup 16SrI-B have been found in association with naturally infected *Daucus carota* L. (carrot), periwinkle and wild *Matricaria chamomilla* L. (chamomile) [34]. Here we report for the first time, to our knowledge, the occurrence of phytoplasmas from the aster yellows group affecting the herbaceous species *Conyza bonariensis* and *Bidens subalternans*. Also, the data provided by *in silico* RFLP profiles and phylogenetic analyses support the description of a novel subgroup within the aster yellows group. A double-letter system has been described to designate new subgroups within the aster yellows group since the letters of the alphabet have been exhausted [13] . Using this system, seven subgroups have been described, 16SrI-AB to 16SrI-AI [13, 36, 37]. According to this, we propose the

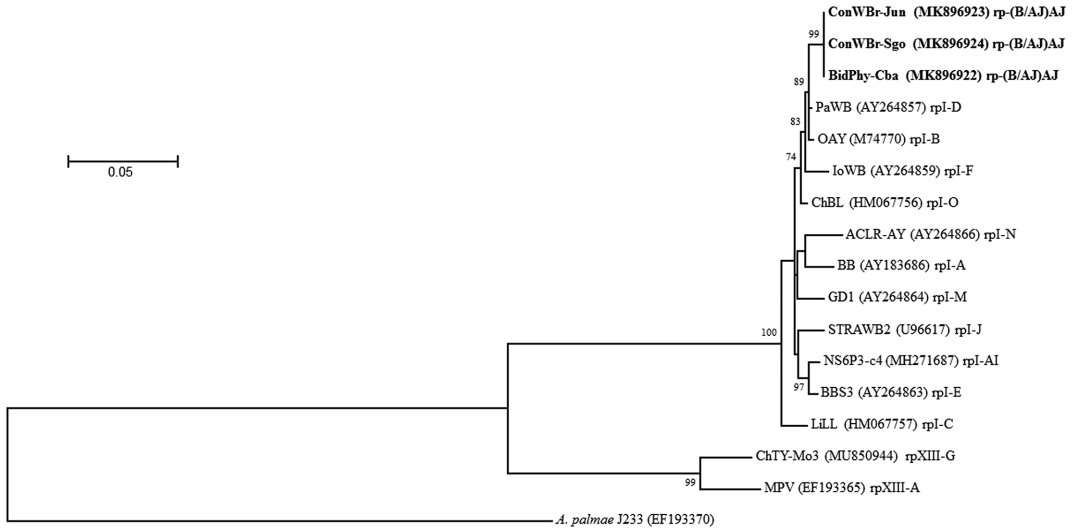

Fig. 4. Phylogenetic relationships inferred from analysis of *rp*-operon gene sequence using the maximum likelihood method implemented in MEGA 6 [23]. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. *Acholeplasma palmae* was used as outgroup. Sequences obtained in this work are in bold type. The corresponding rp-subgroup was added to each taxon.

designation of a novel heterogeneous subgroup 16SrI-(B/AJ)-AJ *rrn*A, *rp*I-AJ.

Three of the plant hosts reported in this work belong to the family *Asteraceae*, one of the largest flowering plant families, which originated in South America [38]. Among them, *Bidens subalternans* and *Heterosperma ovatifolium* are reported as phytoplasma hosts for the first time. *B. subalternans* was infected by phytoplasmas from subgroups 16SrIII-X, 16SrVII-B and the novel subgroup 16SrI-AJ. *Conyza*, another *Asteraceae* genus, represents a suitable host for phytoplasmas since there are several reports of infection by diverse phytoplasmas from the ash yellows (subgroups 16SrVII-B and

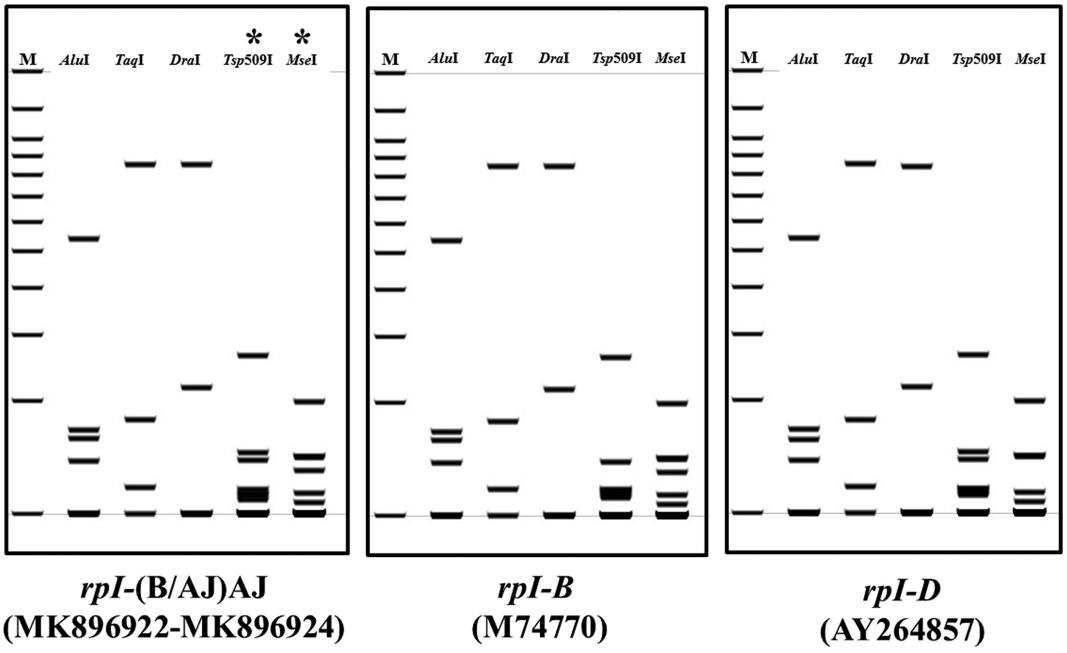

Fig. 5. RFLP patterns derived from *in silico* RFLP analysis of *rp*-operon gene sequence using Geneious R.10 software. *rp*-subgroup reference sequences: *rpl*-B (M74770), *rpl*-D (AY264857) and new *rpl*-(B/AJ) AJ (MK896922MK89694). Differential enzyme patterns are indicated by asterisks. M: 100 bp marker (NEB).

16SrVII-D) [14, 24, 26], X-disease (subgroup 16SrIII-X) [11] and aster yellows groups [36]. Interestingly, plants show similar symptoms, mainly witches' broom, despite being infected by phylogenetically distant phytoplasmas [13]. In this paper, we described a phytoplasma detected in *Conyza bonariensis* and classified it into a novel 16SrI-AJ subgroup, increasing the diversity of phytoplasmas that naturally infect this host. This work contributes to expanding the knowledge about the diversity of the phytoplasmas present in South America, and supports the concept that a unique ecology and geographic separation provided favorable conditions for divergence of phytoplasma lineages from other regions of the world [9].

Weeds play a major role in crop ecosystems. Sometimes, weed plants affected by phytoplasmas are symptomless, probably due to long coevolution between the host and pathogen. If crop plants are grown in the same environment, this natural epidemiological cycle can branch to cultivated plants as dead-end hosts to form a crop-specific epidemic system [39]. In this scenario, there is a need to have a better understanding of the role of weeds both as natural reservoirs of phytoplasmas and in the development of emerging diseases. In this work, diverse and new phytoplasma lineages have been found infecting ever present weeds, which could host potential insect vectors. However, the phytoplasmas described in this paper have not been detected affecting crops in Argentina [8, This paper]. Further investigations related to phytoplasma host specificity, insect vector identification and host plant choice, among others, will led to a better understanding of the effects of this species on different agroecosystems [39, 40].

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
No experiments involving humans or experimental animals were undertaken in relation to this study.

References

1. Lee IM, Davis RE, Gundersen-Rindal DE. Phytoplasma: phytopathogenic mollicutes. *Annu Rev Microbiol* 2000;54:221–255.
2. Bertaccini A, Duduk B, Paltrinieri S, Contaldo N. Phytoplasmas and phytoplasma diseases: a severe threat to agriculture. *Am J Plant Sci* 2014;05:1763–1788.
3. Weintraub PG, Beanland L. Insect vectors of phytoplasmas. *Annu Rev Entomol* 2006;51:91–111.
4. Zhao Y, Davis RE, Wei W, Lee IM. Should '*Candidatus* Phytoplasma' be retained within the order *Acholeplasmatales*? *Int J Syst Evol Microbiol* 2015;65:1075–1082.
5. Lee I, Gundersen-rindal DE, Davis RE, Bartoszyk IM. Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences between distinct groups were 90 % or below. by including additional groups 16S rDNA sequence data were available to predict restriction. *Int J Syst Bacteriol* 1998:1153–1169.
6. Zhao Y, Wei W, Lee I-M, Shao J, Suo X *et al*. Construction of an interactive online phytoplasma classification tool, iPhyClassifier, and its application in analysis of the peach X-disease phytoplasma group (16SrIII). *Int J Syst Evol Microbiol* 2009;59:2582–2593.
7. Naderali N, Nejat N, Vadamalai G, Davis RE, Wei W *et al*. '*Candidatus* Phytoplasma wodyetiae', a new taxon associated with yellow decline disease of foxtail palm (*Wodyetia bifurcata*) in Malaysia. *Int J Syst Evol Microbiol* 2017;67:3765–3772.
8. Conci L, Pons AS, Guzmán F, Fernández F, Galdeano E. Advances in knowledge about phytoplasma diseases in Argentina. In: Bertaccini A (editor). *Phytoplasmas and Phytoplasma Diseases Management: How to Reduce Their Economic Impact*. Bologna: Bologna, Italia; 2014. pp. 82–89.
9. Fernández FD, Marini D, Farrando R, Conci LR. First report of a '*Candidatus* Phytoplasma pyri' strain in Argentina. *Australas Plant Dis Notes* 2017;12:2014–2017.
10. Montano HG, Davis RE, Dally EL, Hogenhout S, Pimentel JP *et al*. '*Candidatus* Phytoplasma brasiliense', a new phytoplasma taxon associated with hibiscus witches' broom disease. *Int J Syst Evol Microbiol* 2001;51:1109–1118.
11. Galdeano E, Guzmán FA, Fernández F, Conci LR. Genetic diversity of 16SrIII group phytoplasmas in Argentina. predominance of subgroups 16SrIII-J and B and two new subgroups 16SrIII-W and X. *Eur J Plant Pathol* 2013;137:753–764.
12. Fernández FD, Galdeano E, Kornowski MV, Arneodo JD, Conci LR. Description of '*Candidatus* Phytoplasma meliae', a phytoplasma associated with chinaberry (*Melia azedarach* L.) yellowing in South America. *Int J Syst Evol Microbiol* 2016;66:5244–5251.
13. Pérez-López E, Luna-Rodríguez M, Olivier CY, Dumonceaux TJ. The underestimated diversity of phytoplasmas in Latin America. *Int J Syst Evol Microbiol* 2016;66:492–513.
14. Meneguzzi NG, Torres LE, Galdeano E, Guzman FA, Nome SF *et al*. Molecular characterization of a phytoplasma of the ash yellows group (16Sr VII-B) occurring in *Artemisia annua* and *Conyza bonariensis* weeds. *AgriScientia* 2008;25:7–15.
15. Catalano MI. Cicadélidos vectores de fitoplasmas a cultivos de importancia económica en la argentina sistemática y bioecología (Insecta- Auchenorrhyncha- Cicadellidae). *Tesis Dr* 2011;134.
16. Fiore N, Longone V, González X, Zamorano A, Pino AM *et al*. Transmission of 16SrIII-J phytoplasma by *Paratanus exitiosus* (Beamer) leafhopper in grapevine. *Phytopathogenic Mollicutes* 2015;5:S43–44.
17. Perilla-Henao LM. *Determination of Phytoplasma Transmission Capacity in Two Morphospecies of the Family Cicadellidae (Hemiptera: Auchenorryncha) from Bogotá, Colombia*. Universidad Nacional de Colombia, PhD Thesis; 2013. p. 156.
18. Kreyci PF, Eckstein B, Lopes JRS, Ferreira J, Bedendo IP. Transmission of "*Candidatus* Phytoplasma pruni"-related strain associated with broccoli stunt by four species of leafhoppers. *J Phytopathol* 2018;166:502–505.
19. Meneguzzi N. Caracterización molecular, taxonomía y diagnóstico de fitoplasmas del grupo Ash Yellows (VII) [PhD thesis]. Tesis Dr, FCEFyN, Universidad Nacional de Córdoba, Córdoba, Argentina; 2009. 147pp.
20. Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. *Focus* 1990;12:13–15.
21. Deng S, Hiruki C. Amplification of 16S rRNA genes from culturable and nonculturable mollicutes. *J Microbiol Methods* 1991;14:53–61.
22. Gundersen D, Lee I. Ultrasensitive by nested-PCR assays detection of phytoplasmas using two universal primer pairs. *Mediterr Phytopathol Union Firenze Univ Press* 1996;35:144–151.
23. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725–2729.
24. Flôres D, Amaral Mello APdeO, Pereira TBC, Rezende JAM, Bedendo IP. A novel subgroup 16SrVII-D phytoplasma identified in association with erigeron witches' broom. *Int J Syst Evol Microbiol* 2015;65:2761–2765.

25. da Silva Fugita JM, Pereira TBC, Banzato TC, Kitajima EW, da Souto ER *et al*. Molecular characterization of a phytoplasma affiliated with the 16SrVII group representative of the novel 16SrVII-F subgroup. *Int J Syst Evol Microbiol* 2017;67:3122–3126.
26. Barros TSL, Davis RE, Resende RO, Dally EL. Erigeron witches'-broom phytoplasma in Brazil represents new subgroup VII-B in 16S rRNA gene group VII, the ash yellows phytoplasma group. *Plant Dis* 2002;86:1142–1148.
27. Pereira TBC, Dally EL, Davis RE, Banzato TC, Galvão SR *et al*. Cauliflower is a new host of a subgroup 16SrVII-B phytoplasma associated with stunting disease in Brazil. *Plant Dis* 2016;100:1007.
28. Pereira TBC, Dally EL, Davis RE, Banzato TC, Bedendo IP. Ming Aralia (*Polyscias fruticose*), a new host of a Phytoplasma subgroup 16SrVII-B strain in Brazil. *Plant Disease* 2016;100:645.
29. Troiani HO, Stiebel PE, Aschemacher A. *Reconocimiento de malezas: región subhúmeda y semiárida pampeana*. Colegio de ingenieros agrónomos de La Pampa. *Santa Rosa. La Pampa. Argentina* 2008.
30. Fernández FD, Conci VC, Kirschbaum DS, Conci LR. Molecular characterization of a phytoplasma of the ash yellows group occurring in strawberry (Fragaria x ananassa Duch.) plants in Argentina. *Eur J Plant Pathol* 2013;135:1–4.
31. Gajardo A, Fiore N, Prodan S, Paltrinieri S, Botti S *et al*. Phytoplasmas associated with grapevine yellows disease in Chile. *Plant Dis* 2009;93:789–796.
32. Franco-Lara L, Contaldo N, Mejia JF, Paltrinieri S, Duduk B *et al*. Detection and identification of phytoplasmas associated with declining *Liquidambar styraciflua* trees in Colombia. *Trop Plant Pathol* 2017;42:352–361.
33. Fernández FD, Meneguzzi NG, Conci LR. Identification of three novel subgroups within the X-disease group phytoplasma associated with strawberry redness disease. *Int J Syst Evol Microbiol* 2017;67:753–758.
34. Torres L, Galdeano E, Fernandez F, Meneguzzi N, Conci L. Establishment of the new subgroup 16SrI-S (rr-rp) tuf-H belonging to 'ca. phytoplasma asteris' in wild and cultivated plants in Argentina. *J Plant Pathol* 2011;93:311–320.
35. Martini M, Lee IM, Bottner KD, Zhao Y, Botti S *et al*. Ribosomal protein gene-based phylogeny for finer differentiation and classification of phytoplasmas. *Int J Syst Evol Microbiol* 2007;57:2037–2051.
36. Jomantiene R, Zhao Y, Lee IM, Davis RE. Phytoplasmas infecting sour cherry and lilac represent two distinct lineages having close evolutionary affinities with clover phyllody phytoplasma. *Eur J Plant Pathol* 2011;130:97–107.
37. Perez-Lopez E, Vincent C, Moreau D, Hammond C, Town J *et al*. A novel '*Candidatus* Phytoplasma asteris' subgroup 16SrI-(E/AI)AI associated with blueberry stunt disease in eastern Canada. *Int J Syst Evol Microbiol* 2018;60:60.
38. Panero JL, Funk VA. The value of sampling anomalous taxa in phylogenetic studies: major clades of the Asteraceae revealed. *Mol Phylogenet Evol* 2008;47:757–782.
39. Duduk B, Stepanovi J, Yadav A, Rao GP. Phytoplasmas in Weeds and Wild Plants. In: Rao G, Bertaccini A, Fiore N and Liefting L (editors). *Phytoplasmas: Plant Pathogenic Bacteria - I*. Singapore: Springer; 2018.
40. Imo M, Maixner M, Johannesen J. Sympatric diversification vs. immigration: deciphering host-plant specialization in a polyphagous insect, the stolbur phytoplasma vector *Hyalesthes obsoletus* (Cixiidae). *Mol Ecol* 2013;22:2188–2203.