'*Candidatus* Phytoplasma tamaricis', a novel taxon discovered in witches'-broom-diseased salt cedar (*Tamarix chinensis* Lour.)

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Salt cedar trees with pronounced witches'-broom symptoms were observed in their natural habitat in China. 16S rRNA gene sequences unique to phytoplasmas were detected in every DNA sample extracted from stem and leaf tissues of the symptomatic trees, revealing a direct association between phytoplasma infection and the salt cedar witches'-broom (SCWB) disease. Phylogenetic analysis of the SCWB phytoplasma 16S rRNA gene sequence indicated that the SCWB phytoplasma belonged to a subclade consisting of several mutually distinct '*Candidatus* Phytoplasma' taxa including '*Ca. Phytoplasma prunorum*', '*Ca. Phytoplasma mali*', '*Ca. Phytoplasma pyri*' and '*Ca. Phytoplasma spartii*'. Pairwise sequence similarity scores calculated from an alignment of near full-length 16S rRNA genes revealed that SCWB phytoplasma shared 96.6% or less sequence similarity with each previously described or proposed '*Ca. Phytoplasma*' taxon, justifying the recognition of SCWB phytoplasma as a novel taxon, '*Candidatus* Phytoplasma tamaricis'. The distinct virtual RFLP pattern derived from the SCWB phytoplasma 16S rRNA gene sequence, together with its lower-than-threshold similarity coefficient values with RFLP patterns of any of the 29 previously established groups, supported the recognition of a new 16Sr group, designated 16SrXXX, salt cedar witches'-broom phytoplasma group.

Phytoplasmas are non-helical, mycoplasma-like cell-wall-less bacteria that are classified in the class *Mollicutes*. Characterized by AT-rich, reductively evolved genomes and a transkingdom parasitic lifestyle, phytoplasmas are known to be pathogenic to more than a thousand plant species and are transmitted by insect vectors, mainly leafhoppers and psyllids (McCoy *et al.*, 1989; Marcone *et al.*, 1999; Lee *et al.*, 2000; Seemüller *et al.*, 2002; Hogenhout *et al.*, 2008). In infected plants, phytoplasmas inhabit the sieve cells of phloem tissue and induce disease symptoms involving disrupted hormonal balance, impaired amino acid and carbohydrate translocation, inhibited photosynthesis and rapid senescence (Chang, 1998; Lepka *et al.*, 1999; Bertamini *et al.*, 2002a, b; Curković-Perica *et al.*, 2007). In their natural insect vectors, phytoplasmas traverse the intestinal wall, circulate in the haemolymph and multiply in diverse tissues including salivary glands,

where phytoplasma cells become incorporated into saliva and are subsequently injected into plants during feeding in the phloem (Seemüller *et al.*, 2002). Phylogenetic studies of genes encoding 16S rRNAs and multiple housekeeping proteins suggest that extant phytoplasmas share a common evolutionary root and are descended from low G+C Gram-positive bacteria in the *Bacillus*–*Clostridium* group (Weisburg *et al.*, 1989; Gundersen *et al.*, 1994; Sears & Kirkpatrick, 1994; Zhao *et al.*, 2005a; Wei *et al.*, 2007, 2008a). Within the monophyletic phytoplasma clade, widely divergent phytoplasma lineages have evolved in adaptation to a broad range of bio- and geo-ecological niches (McCoy *et al.*, 1989; Marcone *et al.*, 1999; Lee *et al.*, 2000; Seemüller *et al.*, 2002; Davis *et al.*, 2005; Wei *et al.*, 2007, 2008b; Cai *et al.*, 2008). Since attempts to isolate and cultivate phytoplasmas in cell-free medium remain unsuccessful, measurable phenotypic characters that are routinely used in microbial classification and taxonomy are essentially inaccessible. To resolve the impediments of phytoplasma taxonomy, the International Research Program for Comparative Mycoplasma, Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group proposed a genus-level provisional taxon '*Candidatus* Phytoplasma', and established guidelines for

Abbreviation: SCWB, salt cedar witches'-broom disease.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of '*Candidatus* Phytoplasma tamaricis' is FJ432664.

The results of PCR amplification from samples of symptomatic salt cedar trees are available as supplementary material with the online version of this paper.

naming 'Ca. Phytoplasma' taxa (IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group, 2004). Thus far, 27 'Ca. Phytoplasma' taxa have been formally described based on 16S rRNA gene sequence phylogeny and/or biological/phytopathological characteristics (IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group, 2004; Firrao *et al.*, 2005; Lee *et al.*, 2006; Valiunas *et al.*, 2006; Arocha, *et al.*, 2007; Al-Saady *et al.*, 2008). In this article, we describe the molecular characterization of a new phytoplasma asso-

ciated with a witches'-broom disease of salt cedar (*Tamarix chinensis* Lour., family *Tamaricaceae*) and propose that the salt cedar witches'-broom (SCWB) phytoplasma be considered as a novel 'Ca. Phytoplasma' taxon.

Association of SCWB disease with phytoplasma infection

Salt cedar is an ornamental shrub/moderate tree species native to Asia and East Europe and was introduced into

Fig. 1. Phylogenetic tree inferred from analysis of 16S rRNA gene sequences. Minimum evolution analysis was conducted using the close neighbour interchange (CNI) algorithm implemented in MEGA4 (Tamura *et al.*, 2007). The initial tree for the CNI search was obtained by the neighbour-joining method. The reliability of the analysis was subjected to a bootstrap test with 1000 replicates. The taxa used in the phylogenetic tree construction included reference strains of 27 previously described 'Ca. Phytoplasma' taxa (labelled with a superscript R), 15 proposed or incidentally cited but yet to be formally described 'Ca. Phytoplasma' taxa (labelled with superscript P) and 'Ca. Phytoplasma tamaricis' (in bold type). *Acholeplasma palmae* served as an outgroup during the phylogenetic tree construction. Bar, number of nucleotide substitutions per site.

Table 1. Phytoplasma strains used in the study

Strain name	GenBank no.	16S RFLP group	Reference
Reference strains of formally described 'Ca. Phytoplasma' taxa			
'Ca. Phytoplasma asteris'	M30790	16SrI-B	Lee <i>et al.</i> (2004a)
'Ca. Phytoplasma aurantifolia'	U15442	16SrII-B	Zreik <i>et al.</i> (1995)
'Ca. Phytoplasma australasiae'	Y10097	16SrII-D	White <i>et al.</i> (1998)
'Ca. Phytoplasma ulmi'	AY197655	16SrV-A	Lee <i>et al.</i> (2004b)
'Ca. Phytoplasma ziziphi' JWB-G1	AB052876	16SrV-B	Jung <i>et al.</i> (2003a)
'Ca. Phytoplasma trifolii'	AY390261	16SrVI-A	Hiruki & Wang (2004)
'Ca. Phytoplasma fraxini'	AF092209	16SrVII-A	Griffiths <i>et al.</i> (1999)
'Ca. Phytoplasma phoenicium'	AF515636	16SrIX-D	Verdin <i>et al.</i> (2003)
'Ca. Phytoplasma mali'	AJ542541	16SrX-A	Seemüller & Schneider (2004)
'Ca. Phytoplasma pyri'	AJ542543	16SrX-C	Seemüller & Schneider (2004)
'Ca. Phytoplasma spartii'	X92869	16SrX-D	Marcone <i>et al.</i> (2004a)
'Ca. Phytoplasma prunorum'	AJ542544	16SrX-F	Seemüller & Schneider (2004)
'Ca. Phytoplasma oryzae'	AB052873	16SrXI-A	Jung <i>et al.</i> (2003b)
'Ca. Phytoplasma australiense'	L76865	16SrXII-B	Davis <i>et al.</i> (1997)
'Ca. Phytoplasma japonicum'	AB010425	16SrXII-D	Sawayanagi <i>et al.</i> (1999)
'Ca. Phytoplasma fragariae'	DQ086423	16SrXII-E	Valiunas <i>et al.</i> (2006)
'Ca. Phytoplasma cynodontis'	AJ550984	16SrXIV-A	Marcone <i>et al.</i> (2004b)
'Ca. Phytoplasma brasiliense'	AF147708	16SrXV-A	Montano <i>et al.</i> (2001)
'Ca. Phytoplasma graminis'	AY725228	16SrXVI-A	Arocha <i>et al.</i> (2005)
'Ca. Phytoplasma caricae'	AY725234	16SrXVII-A	Arocha <i>et al.</i> (2005)
'Ca. Phytoplasma americanum'	DQ174122	16SrXVIII-A	Lee <i>et al.</i> (2006)
'Ca. Phytoplasma castaneae'	AB054986	16SrXIX-A*	Jung <i>et al.</i> (2002); Wei <i>et al.</i> (2007)
'Ca. Phytoplasma rhamni'	X76431	16SrXX-A	Marcone <i>et al.</i> (2004a); Wei <i>et al.</i> (2007)
'Ca. Phytoplasma pini'	AJ632155	16SrXXI-A	Schneider <i>et al.</i> (2005); Wei <i>et al.</i> (2007)
'Ca. Phytoplasma allocasuarinae'	AY135523	Not determined	Marcone <i>et al.</i> (2004a)
'Ca. Phytoplasma lycopersici'	EF199549	Not determined	Arocha <i>et al.</i> (2007)
'Ca. Phytoplasma omanense'	EF666051	16SrXXIX-A†	Al-Saady <i>et al.</i> (2008)
'Ca. Phytoplasma tamaricis'	FJ432664	16SrXXX	Zhao <i>et al.</i> this study
Reference strains of proposed potentially new or incidentally cited taxa			
'Ca. Phytoplasma pruni'	L04682	16SrIII-A	IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group (2004)
'Ca. Phytoplasma palmarum'	U18747	16SrIV-A	IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group (2004)
'Ca. Phytoplasma cocostanzaniae'	X80117	Not determined	IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group (2004)
'Ca. Phytoplasma vitis'	AF176319	16SrV-C	IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group (2004)
'Ca. Phytoplasma luffae'	AF086621	16SrVIII-A	IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group (2004)
'Ca. Phytoplasma solani'	AF248959	16SrXII-A	IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group (2004)
'Ca. Phytoplasma cocosnigeriae'	Y14175	16SrXXII-A	IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group (2004); Wei <i>et al.</i> (2007)
Mexican periwinkle virescence phytoplasma	AF248960	16SrXIII-A	IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group (2004)
Chinaberry yellows phytoplasma	AF495882	Not determined	IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group (2004)
Buckland valley grapevine yellows phytoplasma	AY083605	16SrXXIII-A	Wei <i>et al.</i> (2007)
Sorghum bunchy shoot phytoplasma	AF509322	16SrXXIV-A	Wei <i>et al.</i> (2007)
Weeping tea witches'-broom phytoplasma	AF521672	16SrXXV-A	Wei <i>et al.</i> (2007)
Sugar cane phytoplasma D3T1	AJ539179	16SrXXVI-A	Wei <i>et al.</i> (2007)
Sugar cane phytoplasma D3T2	AJ539180	16SrXXVII-A	Wei <i>et al.</i> (2007)
Derbid phytoplasma	AY744945	16SrXXVIII-A	Wei <i>et al.</i> (2007)

Table 1. cont.

Strain name	GenBank no.	16S RFLP group	Reference
Reference strains of additional 16Sr subgroups			
'Ca. Phytoplasma asteris'-related strain AYWB	NC_007716	16SrI-A	Bai <i>et al.</i> (2006); Lee <i>et al.</i> (1998)
Clover phyllody phytoplasma CPh	AF222065 (<i>rrnA</i>) AF222066 (<i>rrnB</i>)	16SrI-C	GenBank submission in 2001 by Dally, E. L., Bottner, K. D. & Davis, R. E.; Lee <i>et al.</i> (1998)
'Ca. Phytoplasma asteris'-related strain PaWB	AY265206	16SrI-D	GenBank submission in 2004 by Lee, I.-M., Gundersen-Rindal, D. E., Davis, R. E., Bottner, K. D., Marcone, C. & Seemuller, E.; Lee <i>et al.</i> (1998)
Blueberry stunt phytoplasma strain BBS3	AY265213	16SrI-E	GenBank submission in 2004 by Lee, I.-M., Gundersen-Rindal, D. E., Davis, R. E., Bottner, K. D., Marcone, C. & Seemuller, E.; Lee <i>et al.</i> (1998)
'Ca. Phytoplasma asteris'-related strain ACLR-AY	AY265211	16SrI-F	GenBank submission in 2004 by Lee, I.-M., Gundersen-Rindal, D. E., Davis, R. E., Bottner, K. D., Marcone, C. & Seemuller, E.; Lee <i>et al.</i> (1998)
Peanut witches'-broom phytoplasma	L33765	16SrII-A	Lee <i>et al.</i> (1998)
Cactus witches'-broom phytoplasma	AJ293216	16SrII-C	Cai <i>et al.</i> (2002); Wei <i>et al.</i> (2007)
Canada peach X-disease phytoplasma CX	L33733	16SrIII-A	Lee <i>et al.</i> (1998)
Clover yellow edge phytoplasma	AF189288	16SrIII-B	GenBank submission in 1999 by Jomantiene, R., Postman, J. D., Montano, H., Maas, J., Davis, R. E. & Johnson, K. B.; Lee <i>et al.</i> (1998)
Phytoplasma sp. LfY5(PE65)-Oaxaca	AF500334	16SrIV-B	Harrison <i>et al.</i> (2002a); Wei <i>et al.</i> (2007)
<i>Carludovica palmata</i> leaf yellowing phytoplasma	AF237615	16SrIV-D	Harrison <i>et al.</i> (2002b); Wei <i>et al.</i> (2007)
Alder yellows phytoplasma strain ALY882	AY197642	16SrV-C	GenBank submission in 2004 by Lee, I.-M., Martini, M., Marcone, C. & Zhu, S.F.; Lee <i>et al.</i> (1998)
'Ca. Phytoplasma ziziphi'-related strain JWB-Kor1	AB052879	16SrV-G	Jung <i>et al.</i> (2003a); Wei <i>et al.</i> (2007)
Pigeon pea witches'-broom phytoplasma	AF248957	16SrIX-A	Davis & Dally (2001); Lee <i>et al.</i> (1998)

*In the report by Jung *et al.* (2002), '*Ca. Phytoplasma castaneae*' was assigned to group VI according to DNA sequence similarity, rather than results from RFLP analysis. In accordance with the more widely accepted RFLP-based classification system, this phytoplasma was reassigned to group 16SrXIX by Wei *et al.* (2007).

†The original reference (Al-Saady *et al.*, 2008) reported '*Ca. Phytoplasma omanense*' as the reference member of a new group designated group 16SrXIX. However, the group number 16SrXIX had been previously published (Wei *et al.*, 2007) to accommodate a different phytoplasma, '*Ca. Phytoplasma castaneae*'. Therefore, we assign '*Ca. Phytoplasma omanense*' to a new group, 16SrXXIX, subgroup 16SrXXIX-A.

North America during the 1800s (Baum, 1967). Being an Old World species, salt cedar remains a popular, versatile tree in China as well as in other Asian countries, serving ornamental, medicinal and wind-erosion control purposes (Cheng & Zhou, 2001). In contrast, in North America, especially in the western United States, salt cedar has recently been considered as an invasive species that has a negative impact on the ecosystem due to its excessive water consumption and natural predators are being sought to control its spread (Knight, 2001; Gaskin, 2003). In the summer of 2004, salt cedar trees with pronounced witches'-broom symptoms were observed in a suburb of Xi'an City, China (Zhao *et al.*, 2005b). The diseased trees developed dense clusters of thin and highly proliferating twigs. The internodes of the affected twigs were significantly shortened and the scale-like leaves on the affected twigs were greatly reduced in size. These structural abnormalities gave the affected twigs a ball-like appearance.

Phytoplasma infection was suspected as the cause of this SCWB disease because the disease occurred in an area where phytoplasmal diseases, such as paulownia witches'-broom, jujube witches'-broom and wheat blue dwarf have been reported (Kuai *et al.*, 2000). Separate samples of stem/leaf tissues were collected from six branches of two symptomatic salt cedar trees. Total DNA was extracted from each sample using a combined CTAB-DNeasy (Qiagen) method as described by Green *et al.* (1999). The DNA preparations served as templates in six independent PCRs in which phytoplasma-universal primer pair, P1/P7 (Deng & Hiruki, 1991), was used to initiate the amplification reactions. All six reactions yielded an amplicon of approximately 1.8 kb without the need for a nested PCR (see Supplementary Fig. S1 available in IJSEM Online). Four amplicons (two from each diseased plant) were cloned and sequenced on both DNA strands to achieve a 4× coverage per base position. Nucleotide

sequence data revealed that all cloned amplicons were identical and covered a partial rRNA operon, spanning a near full-length 16S rRNA gene, a 16S–23S rRNA intergenic spacer, a tRNA-Ile gene and a partial 23S rRNA gene. The nucleotide sequence was deposited in GenBank (accession no. FJ432664). The 16S rRNA gene portion of the rRNA operon contained a signature sequence, $_{259}5'$ -CAAGACTATGATGTGTAGCTGGACT-3' $_{283}$, that corresponds to a sequence 5'-CAAGAYBA-TKATGKTAGCYGGDCT-3' (IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group, 2004) that is characteristic of phytoplasmas. A BLAST (Altschul *et al.*, 1990) search against the non-redundant nucleotide sequence database in the GenBank (<http://blast.ncbi.nlm.nih.gov/>) using the 1514 bp 16S rRNA gene sequence from the SCWB disease-associated agent as a query returned the most significant matches (with maximum local alignment similarity scores around 96%) to the 16S rRNA genes of several '*Ca. Phytoplasma prunorum*', '*Ca. Phytoplasma mali*'- and '*Ca. Phytoplasma pyri*'-related strains.

The phytoplasma nature of the SCWB disease-associated agent was further confirmed by a phylogenetic analysis of its 16S rRNA gene sequence. A phylogenetic tree was constructed using the minimum evolution approach implemented in the MEGA4 program (Tamura *et al.*, 2007). The topology of the phylogenetic tree (Fig. 1) clearly demonstrated that the SCWB disease-associated agent belonged to the phytoplasma clade and shared a common ancestor with '*Ca. Phytoplasma prunorum*', '*Ca. Phytoplasma mali*', '*Ca. Phytoplasma pyri*', '*Ca.*

Phytoplasma spartii', '*Ca. Phytoplasma rhamni*' and '*Ca. Phytoplasma allocasuarinae*', which, except for '*Ca. Phytoplasma rhamni*', are members of the apple proliferation phytoplasma group (16SrX). '*Ca. Phytoplasma rhamni*' is most closely phylogenetically related to '*Ca. Phytoplasma pyri*' and other fruit tree-infecting phytoplasmas (Marcone *et al.*, 2004a) and was previously classified in the group 16SrX (Lee *et al.*, 1998). Recently, this taxon was assigned to a new 16Sr group (16SrXX) on the basis of its lower-than-threshold RFLP pattern similarity coefficient values with all known phytoplasmas in group 16SrX and other groups (Wei *et al.*, 2007) in accordance with the principle that governs the 16S rRNA gene sequence RFLP-based classification scheme (Lee *et al.*, 1998). Based on the symptoms it produces in its plant host, the signature sequence of its 16S rRNA gene and its phylogenetic position, the SCWB disease-associated agent is hereby designated SCWB phytoplasma, strain SCWB1.

SCWB1 represents a novel taxon

For overall sequence comparison and pairwise sequence similarity score calculation, a global sequence alignment was constructed using the CLUSTAL W option of the LASERGENE MEGALIGN program (DNASTAR). The alignment included 16S rRNA gene sequences from SCWB1, from reference strains of all 27 formally described '*Ca. Phytoplasma*' taxa and from reference strains of 15 proposed, new but yet to be formally described '*Ca. Phytoplasma*' taxa (Table 1). Exhaustive comparisons of the aligned sequences identified regions unique to the SCWB1 16S rRNA gene. These distinguishing regions include $_{167}5'$ -ATTAGGCATCTAGTAACTTTG-3' $_{187}$, $_{597}5'$ -TGCTCAACATTGTTGC-3' $_{612}$, $_{971}5'$ -AGCTTTGCAAAGT-TG-3' $_{985}$, and $_{994}5'$ -TAACAGAGGTTATCAGAGTT-3' $_{1013}$, which differ in from five to eleven, from three to six, from four to eight and from four to nine base positions, respectively, from the corresponding regions in the 16S rRNA genes of all previously described or proposed '*Ca. Phytoplasma*' taxa (the original alignment file is available from the authors upon request). Pairwise sequence similarity scores revealed that the 1514 bp near-full-length SCWB1 16S rRNA gene shared no greater than 96.6% nucleotide sequence similarity with any of the 27 formally described and 15 proposed but yet to be formally described '*Ca. Phytoplasma*' taxa (incidental citations). According to recommendations by the IRPCM, Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group (IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group, 2004), 'a strain can be described as a novel '*Ca. Phytoplasma*' [candidate] species if its 16S rRNA gene sequence has <97.5% similarity to that of any previously described '*Ca. Phytoplasma*' [candidate] species'. Results from our analysis of the SCWB1 16S rRNA gene, together with the natural host and geographical information, justify the recognition of the SCWB phytoplasma as a novel taxon. Thus, we propose that the phytoplasma strain

Fig. 2. RFLP profiles derived from 16S rRNA gene F2nR2 fragments of '*Ca. Phytoplasma tamaricis*' representative strain SCWB1 (16SrXXX-A). (a) Virtual RFLP patterns derived from *in silico* digestions using 17 restriction enzymes: *AluI*, *BamHI*, *BfaI*, *BstUI* (*ThaI*), *DraI*, *EcoRI*, *HaeIII*, *HhaI*, *HinfI*, *HpaI*, *HpaII*, *KpnI*, *Sau3AI* (*MboI*), *MseI*, *RsaI*, *SspI* and *TaqI*. (b) Actual restriction patterns derived from digestions using three distinguishing enzymes: *AluI*, *BfaI* and *MseI*. The restriction fragments were resolved by 3% agarose gel electrophoresis. MW, ϕ X174DNA-*HaeIII* digestion.

Fig. 3. Distinguishing restriction enzymes that produce unique restriction patterns of the SCWB1 rRNA gene. Subgroup pattern 16SrXXX-A can be distinguished from all other pattern types by *AluI* (a), *BfaI* (b) or *MseI* (c) single enzyme digestion. M, ϕ X174DNA-*HaeIII* digestion.

SCWB1 be designated the reference strain of a distinct '*Ca. Phytoplasma*' taxon, '*Candidatus Phytoplasma tamaricis*'.

SCWB1 represents a new and distinct 16Sr RFLP group

In parallel to '*Candidatus*' taxon assignment, phytoplasmas are often classified into groups on the basis of RFLP patterns derived from digestion of the 16S rRNA gene F2nR2 fragment by a set of 17 restriction enzymes, a classification scheme established by Lee *et al.* (1993). Through periodic updates (Lee *et al.*, 1998, 2000, 2006) and recent expansions through computer-simulated RFLP analysis (Wei *et al.*, 2007, 2008b; Cai *et al.*, 2008), this 16S rRNA gene RFLP-based classification scheme has accommodated 29 groups (16SrI to 16SrXXIX). Such RFLP pattern-based group classification has provided 'visible' molecular markers for rapid and reliable identification of a

broad array of phytoplasmas. In this classification system, the key criterion for the delineation of new 16Sr groups has been the threshold RFLP pattern similarity coefficient value, which was initially set at 0.9 (Lee *et al.*, 1998) and was recently modified to 0.85 (Wei *et al.*, 2007) to reflect precisely the existing group-level classification status and to guide future recognition of new groups. A new group is recognized if the RFLP pattern derived from a given phytoplasma strain exhibits a similarity coefficient value of lower than 0.85 with the RFLP patterns of all other previously recognized groups.

Virtual RFLP analysis of the '*Ca. Phytoplasma tamaricis*' SCWB1 16S rRNA gene F2nR2 fragment revealed a new RFLP pattern (Fig. 2a). The new pattern was confirmed by an actual enzymic RFLP analysis using three distinguishing enzymes *AluI*, *BfaI* and *MseI* (Fig. 2b and Fig. 3). This new pattern had similarity coefficient values lower than 0.77 with the RFLP pattern types of any previously established

groups (the complete similarity coefficient table is available from the authors on request). The distinct 16S rRNA gene RFLP pattern and the lower-than-threshold similarity coefficient values exhibited by the SCWB1 phytoplasma clearly support the recognition of a new 16Sr group, which is hereby designated salt cedar witches'-broom phytoplasma group, 16SrXXX. Erection of this new group is also consistent with the proposal (original group concept) that each 16Sr group represents at least one species (Lee *et al.*, 1998).

Since, at the present time, SCWB1 is the only strain in group 16SrXXX, it becomes the representative strain of subgroup 16SrXXX-A. The unique 16SrXXX-A pattern can be readily distinguished from all other previously delineated 16Sr group/subgroup patterns by *AluI*, *BfaI* or *MseI* single restriction enzyme digestion (Fig. 3).

Description of 'Candidatus Phytoplasma tamaricis'

'Candidatus Phytoplasma tamaricis' (tam.ar.i.cis. N.L. gen. n. *tamaricis* of *tamarix*, the scientific name of salt cedar; the epithet referring to the plant host).

Reference strain is SCWB1^R.

[(*Mollicutes*) NC; NA; O, wall-less; NAS (GenBank accession number FJ432664), oligonucleotide sequences of unique regions of the 16S rRNA gene 5'-ATTAGG-CATCTAGTAACCTTG-3', 5'-TGCTCAACATTGTTGC-3', 5'-AGCTTTGCAAAGTTG-3' and 5'-TAACAGAGG-TTATCAGAGTT-3'; P (*Tamarix chinensis*, phloem); M]. Zhao *et al.*, this study.

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