‘Candidatus Phytoplasma pruni’, a novel taxon associated with X-disease of stone fruits, Prunus spp.: multilocus characterization based on 16S rRNA, secY, and ribosomal protein genes

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X-disease is one of the most serious diseases known in peach (Prunus persica). Based on RFLP analysis of 16S rRNA gene sequences, peach X-disease phytoplasma strains from eastern and western United States and eastern Canada were classified in 16S rRNA gene RFLP group 16SrIII, subgroup A. Phylogenetic analyses of 16S rRNA gene sequences revealed that the X-disease phytoplasma strains formed a distinct subclade within the phytoplasma clade, supporting the hypothesis that they represented a lineage distinct from those of previously described ‘Candidatus Phytoplasma’ species. Nucleotide sequence alignments revealed that all studied X-disease phytoplasma strains shared less than 97.5% 16S rRNA gene sequence similarity with previously described ‘Candidatus Phytoplasma’ species. Based on unique properties of the DNA, we propose recognition of X-disease phytoplasma strain PX11CT1R as representative of a novel taxon, ‘Candidatus Phytoplasma pruni’. Results from nucleotide and phylogenetic analyses of secY and ribosomal protein (rp) gene sequences provided additional molecular markers of the ‘Ca. Phytoplasma pruni’ lineage. We propose that the term ‘Ca. Phytoplasma pruni’ be applied to phytoplasma strains whose 16S rRNA gene sequences contain the oligonucleotide sequences of unique regions that are designated in the formally published description of the taxon. Such strains include X-disease phytoplasma and - within the tolerance of a single base difference in one unique sequence - peach rosette, peach red suture, and little peach phytoplasmas. Although not employed for taxon delineation in this work, we further propose that secY, rp, and other genetic loci from the reference strain of a taxon, and where possible oligonucleotide sequences of unique regions of those genes that distinguish taxa within a given 16Sr group, be incorporated in emended descriptions and as part of future descriptions of ‘Candidatus Phytoplasma’ taxa.

Peach (Prunus persica) and other stone fruit tree species are affected by diseases that seriously impact agricultural production. X-disease has historically been one of the major limiting factors in peach production in the United States (Stoddard et al., 1951). First reported in 1933 in the state of Connecticut and called the ‘X disease of peach’ because of its unknown cause and mysterious nature, X-disease of peach was for many years believed to be caused by a virus (Stoddard, 1934, 1938; Stoddard et al., 1951). Extensive early research indicated that the presumed virus was seldom passed from peach tree to peach tree in the eastern US, but instead was mainly carried by insect vectors from nearby forest growth to peach orchards. An important natural plant host of the pathogen was found to be wild chokecherry (Prunus virginiana) growing in the forest (reviewed by Douglas, 1986). In ensuing years, X-disease was reported in several states in the north-eastern US, in California and other western states, and in Canada.

Abbreviations: hf-PCRs, high-fidelity polymerase chain reaction; RFLP, restriction fragment length polymorphism.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, secY, and ribosomal protein (rp) gene sequences of strain PX11CT1R are JQ044393 (rrnA), JQ044392 (rrnB), JQ268254 (secY), and JQ360960 (rp), respectively. The GenBank/EMBL/DDBJ accession numbers for other strains used in this study are listed in Table 1.

A supplementary figure and a supplementary table are available with the online version of this paper.
(Stoddard et al., 1951). Studies in eastern and western US have revealed that the pathogen can be transmitted by species of leafhopper, including Colladonas citellarius, C. montanus, C. geminatus, Euscelidius variegatus, Fieberiella florii, Graphocephala confluens, Gyponana lamina, Kerneilla confluens, Norvellina seminuda, Osbornellus borealis, Paraphlepsius irroratus, and Scaphytopius delongi (S. acutus) (Kirkpatrick et al., 1990; Larsen & Whalen, 1988; McClure, 1980; Rice & Jones, 1972). In addition to peach and chokecherry, species of the genus Prunus that are reportedly susceptible to infection by the X-disease pathogen include cherry (Prunus avium and Prunus cerasus), Japanese plum (Prunus salicina), almond (Prunus dulcis), apricot (Prunus armeniaca), nectarine (Prunus persica var. nectarina), Chinese bushcherry (Prunus japonica), Bessey cherry (Prunus besseyi), wild American plum (Prunus americana), wildgoose plum (Prunus munsoniana), and European plum (Prunus domestica) (Douglas, 1986; Stoddard et al., 1951).

Symptoms of X-disease on peach include tattered, shot-holed appearance of leaves; loss of severely affected leaves, leaving a cluster of leaves at the ends of individual branches; dieback of branches; and death of trees (Douglas, 1986; Stoddard, 1938; Stoddard et al., 1951). Application of insecticides in an effort to stem the spread of X-disease, destruction of nearby chokecherry, treatment of trees with tetracycline antibiotic, removal of symptomatic branches from diseased trees, replacement of trees that are killed by the disease, and an integrated approach to disease control have been practiced with varying degrees of success (Allen & Davidson, 1978; Amin & Jensen, 1971; Cooley et al., 1992; Douglas & McClure, 1988; Lacy, 1982; Lacy et al., 1980; Lukens et al., 1971; McClure et al., 1982; Nyland, 1971; Pearson & Sands, 1978; Rosenberger & Jones, 1977; Sands & Walton, 1975).

X-disease of stone fruits is now attributed to infection of plants by a phytoplasma (formerly mycoplasma-like organism; MLO), a wall-less bacterium embraced taxonomically within the class Mollicutes (Douglas, 1986; Granett & Gilmer, 1971; Jones et al., 1974; Lee et al., 1992; MacBeath et al., 1972; Nasu et al., 1970). In their evolutionary descent from walled bacteria in the Bacillus/ Clostridium group, phytoplasmas underwent extensive genome size reduction (Marcone et al., 1999; Oshima et al., 2004), losing genes that became unnecessary during adaptations to obligate, intracellular parasitism in plant and insect hosts (Davis et al., 2005). Due to the consequent inability to obtain pure cultures of phytoplasmas in vitro, properties used to distinguish and describe species of cultivable bacteria, including cultivable mollicutes, are largely unattainable for phytoplasmas. However, DNA-based technologies have enabled considerable progress in phytoplasma classification and taxonomy.

Over the past two decades, advances in DNA-based technologies have led to the establishment of a group/subgroup classification system based on restriction fragment length polymorphism (RFLP) analysis of 16S rRNA gene sequences, the characterization and phylogenetic analysis of numerous phytoplasma strains, the establishment of a provisional ‘Candidatus Phytoplasma’ taxonomy by the International Research Program for Comparative Mycoplasmology Phytoplasma/Spiroplasma Working Team-Phytoplasma Taxonomy Group (IRPCM, 2004), and the formal description of over 30 ‘Candidatus Phytoplasma’ taxa (Davis et al., 2012; Gundersen et al., 1994; IRPCM, 2004; Lee et al., 1998; Martini et al., 2012). The phytoplasma strains that are associated with X-disease are classified in phytoplasma 16S rRNA gene RFLP group (16Sr group) 16SrIII, subgroup 16SrIII-A. Related strains, classified in other subgroups of group 16SrIII, are the apparent causes of diseases in diverse plant species, including blueberry, clover, goldenrod, milkweed, spirea, poinsettia, potato, walnut, and pecan (Table 1). The name ‘Candidatus Phytoplasma pruni’ was informally suggested for X-disease phytoplasma (IRPCM, 2004), but the taxon was not formally described prior to the present communication.

In this communication, we report results from the study of phytoplasma strains associated with X-disease of peach in the United States and Canada, and of phytoplasma strains associated with peach rosette, little peach, and peach red suture diseases in the United States. In the present study, samples of symptomatic leaves were collected in 1992 and 2011 from a total of six naturally diseased, commercially cultivated peach trees exhibiting symptoms characteristic of X-disease in the state of Connecticut, United States. The symptoms on the peach trees included chlorosis and brittleness of leaves, tattered appearance of leaves (Fig. 1a), premature leaf drop, and dieback of branches. The collected leaves resembled peach X-disease symptomatic leaves depicted by Stoddard (1938) (Fig. 1b). Phytoplasma strains Canada X-disease (CX) and western X-disease (WX) were each maintained separately by grafting in plants of [periwinkle] (Catharanthus roseus); Clover yellow edge phytoplasma strain CYE (CYE-C) was in clover (Trifolium repens); and Walnut witches'-broom (WWB) phytoplasma was in lyophilized tissue of a WWB-diseased walnut tree. Nucleic acid for use as template in PCR was extracted from peach tissue samples, each consisting of mid-veins excised from symptomatic leaves collected from one of the six diseased peach trees: DNA was extracted from leaves collected from two peach trees in 2011 by the use of a modified method (Green et al., 1999) using DNeasy Plant DNA Extract kit (Qiagen); DNA was extracted in 1993 from leaves collected from four peach trees in 1992 using methods described by Prince et al. (1993). Template DNAs from walnut witches'-broom (WWB) and X-disease phytoplasma strains WX (WX95) and CX (CX-95) were extracted from diseased plant tissues as described previously (Lee et al., 1991). Template DNAs were stored at −20°C until further use.

For analysis of 16S rRNA gene sequences, the DNA templates were used in separate, direct high-fidelity PCRs (hf-PCRs) primed by primer pair P1/16S-SR and catalysed
by AccuPrime Taq DNA Polymerase High Fidelity (Life Technologies) under conditions after Lee et al. (2004). To confirm the presence of sequence-heterogeneous rRNA operons, products of PCR primed by P1/16S-SR were subjected to restriction endonuclease digestion, and the resulting digest was analysed by 1% agarose gel electrophoresis. For analysis of ribosomal protein (rp) genomic regions, DNA segments were amplified in hf-PCRs primed by primer pair rpL2F3/rp(II)R1A in direct PCR (for strain CX-95), and in nested PCRs primed by rpL2F3/rp(II)R1A (Martini et al., 2007) followed by rp(III)-FN/rp(II)R1A (for the remaining strains) under conditions after Martini et al. (2007). The nucleotide sequence of primer rp(III)-FN was 5’-GGTGAATTTCCTCCACTCG-3’ (this study). For analysis of secY genomic regions from studied strains (except for strain CX-95), DNA fragments were amplified in hf-PCRs primed by L15F1A(III)/MapR1A(III) and catalysed by LA Taq DNA Polymerase (Takara Bio USA), followed by nested hf-PCR primed by secYF1(III)/secYR1(III) and catalysed by AccuPrime Taq DNA Polymerase High Fidelity under

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*16S rRNA gene RFLP group and subgroup classification of phytoplasma strain. Group is indicated by a Roman numeral; subgroup is indicated by a letter. Strain variant within a designated subgroup is indicated by an asterisk.
†Phytoplasma strains first reported in this study.
‡Nucleotide sequences determined in this study. For strains in which two sequence heterogeneous 16S rRNA genes were found, the identity of each partially sequenced rRNA operon is in parentheses.
conditions previously described (Lee et al., 2010; Martini et al., 2007). SecY genomic regions from strain CX-95 were amplified in direct hf-PCRs primed by primer pair SecYF1(III)/SecYR1(III). A negative control devoid of DNA template in the reaction mix was included in all PCR assays.

PCR products representing amplified genomic regions were analysed by 1% agarose gel electrophoresis. The phytoplasma-specific DNA bands were prepared for sequencing by using a QIAquick Gel Extraction kit (Qiagen). The purified, amplified genomic regions were then sequenced by automated DNA sequencing (ABI Prism model 3730) of both strands to achieve 36 to 56 coverage per base position. Nucleotide sequences of genes from other phytoplasmas in this study were obtained from the GenBank database (http://www.ncbi.nlm.nih.gov/) (Table 1 and Table S1, available in IJSEM Online). Virtual RFLP analysis of nucleotide sequences and determination of 16Sr group and subgroup affiliations were performed using iPhyClassifier (Wei et al., 2007; Zhao et al., 2009; http://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclasifier.cgi). Phylogenetic analyses and nucleotide sequence alignments were performed using CLUSTAL in the MEGA4 software (Tamura et al., 2007) and CLUSTAL version 5 from the LaserGene MEGALIGN program (DNASTAR). Oligonucleotide sequences of unique regions in the 16S rRNA gene of ‘Ca. Phytoplasma pruni’ were selected such that they were identical in both rRNA operons of the reference strain.

In repeated experiments, 1.6 kb 16S rRNA gene fragments were amplified by PCR with primers P1/16S-SR, indicating that all six peach trees exhibiting symptoms of X-disease in Connecticut were infected by phytoplasma. The phytoplasmas detected in the six trees were designated strains PX11CT1R and PX11CT2 (strains found in two trees sampled in 2011), and strains PX92CT1, PX92CT2, PX92CT3, and PX92CT4 (strains found in four trees sampled in 1992), respectively.

Sequence-heterogeneous 16S rRNA genes in X-disease phytoplasma strains

Examination of the chromatograms from nucleotide sequencing of 16S rRNA gene amplicons from the Connecticut X-disease phytoplasma strains revealed that a single base position in the 16S rRNA gene (corresponding to position 838 in GenBank accession no. JQ044393 of strain PX11CT1R) was represented by two base-call peaks, one indicating a base call C and the other indicating a T, yielding a consensus Y, whereas all other base positions were each represented by single peaks (Fig. 2a and data not shown). We hypothesized that this result indicated the presence of two amplicons that were derived from two sequence-heterogeneous rRNA operons in each of the strains from Connecticut. To test this hypothesis, we digested the 16S rRNA gene products, from PCRs primed by P1/16S-SR, with the restriction endonuclease HpaI, since the C in one of the hypothesized operons was located within this enzyme’s recognition site (GTTAAC) in the 16S rRNA gene. The resulting RFLP profiles after gel electrophoresis of the digested DNA (Fig. 2b) clearly indicated the presence of two 16S rRNA gene species in each PCR product, consistent with one undigested 16S rRNA gene amplicon containing a T and a digested 16S rRNA gene amplicon containing a C in the region corresponding to the HpaI site. This finding, and cloning and sequencing of the two 16S rRNA gene species, confirmed that the automated sequencing had revealed two sequence-heterogeneous 16S rRNA genes in the PCR products from the peach X-disease phytoplasma strains from Connecticut. By contrast, neither the automated sequencing chromato-

![Symptomatic leaves from X-diseased peach trees (Prunus persica) in Connecticut.](http://ijs.sgmjournals.org)
grams, nor digestion of PCR products with HpaI, indicated the presence of sequence-heterogeneous 16S rRNA genes in strains CX-95 or WX95 (Fig. 2b and WX data not shown), consistent with the concept that these strains and the Connecticut strains possibly represent two closely related lineages. Nucleotide sequences of the 16S rRNA gene sequences from strains CX-95 and WX95, and from the two sequence-heterogeneous rRNA operons of each Connecticut X-disease phytoplasma strain, were deposited in the GenBank database (Table 1).

**16Sr group and subgroup classification of X-disease and other peach-associated strains**

Sequence alignments revealed that strains PX11CT1R, PX11CT2, PX92CT1, PX92CT2, PX92CT3, and PX92CT4 shared 100% 16S rRNA gene sequence similarity, of corresponding rRNA operons, with one another. As expected, results from computer-simulated, virtual RFLP analysis of 16S rRNA gene sequences revealed that all of these X-disease strains are members of group 16SrIII, subgroup 16SrIII-A, yielding 16S rRNA gene RFLP profiles identical with those of the two rRNA operons, rrnA and rrnB, of strain PX11CT1R (Fig. S1). Results from virtual RFLP analysis of 16S rRNA gene sequences of little peach and peach red suture phytoplasmas indicated that these phytoplasmas are also members of subgroup 16SrIII-A, and that peach rosette phytoplasma may be a subgroup 16SrIII-A variant strain whose 16S rRNA gene differs by a single base substitution in a Sau3AI restriction enzyme site (data not shown). Strain PX11CT1R from peach in Connecticut was selected for further study as representative of a novel taxon.

**Novel taxon from X-diseased peach**

To determine whether strain PX11CT1R represented a distinct taxon, the MEGALIGN option of the DNASTAR program and iPhyClassifier was used to align 16S rRNA gene sequences and calculate sequence similarities with 16S rRNA gene sequences of other phytoplasmas. The 16S rRNA gene sequences from both rRNA operons of strain PX11CT1R exhibited less than 97.5% sequence similarity with the corresponding fragments of 16S rRNA gene sequences from all previously described ‘Ca. Phytoplasma’ species. Therefore, in accordance with guidelines for the recognition of ‘Candidatus Phytoplasma’ species (IRPCM, 2004), we propose that strain PX11CT1R is representative of a novel, distinct ‘Candidatus Phytoplasma’ taxon, designated ‘Candidatus Phytoplasma pruni’.

A phylogenetic tree was constructed based on 16S rRNA gene sequences from all previously described ‘Ca. Phytoplasma’ taxa, the proposed ‘Ca. Phytoplasma pruni’, and Acholeplasma palmae; the two sequence-heterogeneous 16S rRNA genes of ‘Ca. Phytoplasma pruni’ were included (Table S1). The phylogenetic analysis indicated that ‘Ca. Phytoplasma pruni’ formed a well-supported branch representing a distinct, taxon level lineage (Fig. 3).

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**Fig. 2. Evidence of sequence heterogeneous 16S rRNA genes in X-disease phytoplasma.** (a) Chromatogram from nucleotide sequencing of PCR-amplified rRNA gene amplicons from strain PX11CT1R. Double base-call is indicated by two peaks representing bases C and T, respectively. The recognition site for restriction enzyme HpaI is underlined; the location of enzymic cutting is indicated by a solid triangle. (b) RFLP patterns before (U) and after (D) HpaI digestion of DNA. PX1, strain PX11CT1R, indicating two sequence-heterogeneous rRNA operons. PX2, strain PX11CT2, indicating two sequence-heterogeneous rRNA operons. CX, strain CX-95, without evidence for sequence-heterogeneous rRNA operons. S, size standard 1 kb DNA ladder (Life Technologies).
Fig. 3. Phylogenetic tree inferred from analyses of 16S rRNA gene sequences from ‘Candidatus Phytoplasma pruni’ species. The taxa used in construction of the phylogenetic tree included reference strains of 32 previously described ‘Ca. Phytoplasma’ species and ‘Ca. Phytoplasma pruni’ proposed in this communication (in bold type). Acholeplasma palmae ATCC 49389 (L33734) served as the outgroup. GenBank accession numbers are given in parentheses. Maximum-parsimony analysis was conducted using the close neighbour interchange (CNI) algorithm implemented in software package MEGA4. The initial tree for the CNI search was obtained with the random addition of sequences (10 replicates). The reliability of the analysis was subjected to a bootstrap test with 1000 replicates. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches. Bar, 10 nt substitutions.

Description of ‘Candidatus Phytoplasma pruni’

‘Candidatus Phytoplasma pruni’ (pru‘ni. L. gen. n. pruni of a peach-tree, from a tree of the genus Prunus).

PX11CT1R is the reference strain.

[(Mollicutes) NC; NA; O, wall-less; NAS (GenBank accession no. JQ044393, rrnA; and JQ044392, rrnB), oligonucleotide sequences of unique regions in the 16S rRNA gene are 5’-CACATTAGTTAGTGGTAGTTAAAGGCCCCTAC-C-3’ (226-258), 5’-TACCTGCTATGTAAT-3’ (402-414), 5’-TATTAAGGAAGAAAAAGAGTGGAAGAAGACTCCCTT-3’ (425-459), 5’-ACGGTACTTAA-3’ (462-472), 5’-TAAT-AAGTCTATAGTTAAATTTCCAGCTGGAAGTGTGTTG- GCTATAG-3’ (571-618), 5’-GTGGTTACTAGTGAG-3’ (624-639), 5’-TAAAACCTGGTAC-3’ (817-828), 5’-TTCT-GCGAAGTTA-3’ (970-984), 5’-ATGGAGGTCATCAGG-AAAACAGGTTGTCG-3’ (999-1027), 5’-CTTGTCTTGTATGGCCGCACTGTAAT-3’ (1083-1108), 5’-GATGGGGGACTTTAACCAG-3’ (1109-1125), 5’-GGTTGATACAAAG-3’ (1211-1223), and 5’-TCTCAAAAAATCAATC-3’ (1252-1267); P (Prunus, phloem); MJ. Davis et al., this study.

Ribosomal protein (rp) and secY loci in ‘Ca. Phytoplasma pruni’ and related strains

‘Ca. Phytoplasma pruni’ PX11CT1R and additional X-disease phytoplasma strains were further distinguished from other phytoplasmas of group 16SIII, on the basis of analyses of genomic segments containing genes encoding ribosomal proteins and preprotein translocase SecY subunit.

In direct PCR, primer pair rpL2F3/rp(I)R1A primed amplification of a 1.7 kb DNA fragment, containing genes

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between the strains in this study lacked an (methionine aminopeptidase) gene. Like various group a plasma strain clusters, one group containing strains having genomic locus distinguished two group 16SrIII phytoplasma subclades. A 9-base insertion/deletion (indel) in the major evolutionary divergence among phytoplasma subgroups in group 16SrIII strains studied, CYE phytoplasma has a 3-base deletion and a 7-base insertion, the latter as part of a possibly lineage-specific 7-base direct repeat, in the gene-encoding L22, and a 3-base insertion in the gene-encoding S3.

Direct and nested PCRs with primer pair secYF1(III)/secYR1(III) yielded 1.7 kb DNA fragments from X-disease phytoplasma DNA templates (Table 1). Nucleotide sequence analyses revealed that the amplicons each contained a partial (3’-end) rpl15 ribosomal protein (rp) gene, a complete secY gene, and a partial (5’-end) map (methionine aminopeptidase) gene. Like various group 16SrIII phytoplasma strains and unlike some other phytoplasmas (Lee et al., 2010), the X-disease phytoplasma strains in this study lacked an adk (adenylate kinase) gene between the secY and map genes, a feature that marks a major evolutionary divergence among phytoplasma subclades. A 9-base insertion/deletion (indel) in the secY genomic locus distinguished two group 16SrIII phytoplasma strain clusters, one group containing strains having a secY gene length of 1263 bases (strains PX11CT1R, PX11CT2, PX92CT1, PX92CT4, CX-95, WX95, WWB, PoiBI, SP1), and the other group containing strains having secY length of 1272 bases (strains CYE, MW1, GR1, PBT, PPTAKpot6, PPTAKpot7, PPTMT117, VAC) (Fig. 4). Single nucleotide polymorphisms (SNPs) further distinguished the X-disease strains from group 16SrIII strains that are associated with other diseases and are classified in other than subgroup 16SrIII-A (Fig. 4 and data not shown).

Alignment of deduced amino acid sequences revealed that, due to the observed 9-base indel, the SecY proteins encoded by X-disease, PoiBI, WWB, and SP1 phytoplasma strains shared a unique amino acid sequence including a three-amino acid deletion, in the context KQY—FS, compared to the corresponding region [KQ(K/E)LFKL(S/Y)] in group 16SrIII strains VAC, GR1, AKpot6, AKpot7, CYE, MT117, MW1, and PBT. Interestingly, the secY-map intergenic region from all of the X-disease phytoplasma strains and strain PoiBI contained a 4-base insertion (bases TTTG at positions 1625–1628 in GenBank sequence JQ044393, strain PX11CT1R), compared to the secY-map intergenic regions from strains affiliated with diverse subgroups of group 16SrIII (Fig. 5).

Phylogenetic analyses of 16S rRNA, secY, and ribosomal protein gene sequences yielded three mutually congruent trees, on each of which ‘Ca. Phytoplasma prunii’ PX11CT1R and other X-disease-associated strains formed a well-supported, distinct cluster (Fig. 6). The topologies of the secY and rp gene trees suggested at least two divergent branches of evolution, with strain CYE possibly representing a third (Fig. 6). The results were consistent with the concept that the X-disease strains represented a lineage that was distinct among diverse subgroups within group 16SrIII and raised the possibility that the group 16SrIII phytoplasma lineages analysed represented at least two and perhaps at least three different phytoplasma taxa. In particular, we noted that strain CYE was distinguished from the other phytoplasma strains in this study, by its phylogenetic position in the rp and secY gene trees.

According to guidelines proposed by the IRPCM (2004), the term ‘Candidatus Phytoplasma prunii’ would strictly be applied only to the reference strain (PX11CT1R) described herein, and other strains of X-disease phytoplasma would be referred to as ‘Candidatus Phytoplasma prunii’-related strains. Thus under this convention, multiple strains undoubtedly of a given taxon, such as ‘Candidatus Phytoplasma prunii’, are referred to simply as related to that taxon, potentially implying that such strains may represent separate taxon level lineage(s). Other members of group 16SrIII, including strains classified in diverse subgroups in group 16SrIII, would also be referred to as ‘Candidatus Phytoplasma prunii’-related strains unless named as separate taxa. Thus, more distantly related strains, that may well represent distinct taxon level lineage(s), are disadvantageously not distinguished as such.

To resolve this issue, we propose that the term ‘Candidatus Phytoplasma prunii’ be applied to all strains whose 16S rRNA gene sequences contain the oligonucleotide sequences of unique regions that are designated in the formally published description of the taxon (this study) and as may be emended and/or revised. Results from nucleotide sequence alignments indicated that application of this guideline would include geographically diverse X-disease phytoplasma strains within ‘Ca. Phytoplasma prunii’, but would exclude group 16SrIII strains such as clover yellow edge, potato purple top, walnut witches’-broom, and other phytoplasmas listed in Table 1. In our view, the term ‘Ca. Phytoplasma prunii’-related strains would be applied to such latter phytoplasmas (Table 1). In these latter strains, the 16S rRNA genes differed by one or more bases in two or more of the oligonucleotide sequences that correspond to unique regions designated
of phytoplasma species descriptions, because evolutionary revisions of species-unique oligonucleotide sequences in 16S of existing 'Candidatus Phytoplasma pruni' (data not shown) are present in the remaining portion of the gene encoding 16S rRNA (Davis & Sinclair, 1998). In this communication, we propose recognition of a new species, 'Candidatus Phytoplasma pruni', principally on the basis of the gene encoding 16S rRNA. While current guidelines recommend analysis and description of only this single gene (from just one rRNA operon) for delineation of such a unique 'Candidatus Phytoplasma' species (IRPCM, 2004), we include nucleotide sequences for both sequence-heterogeneous 16S rRNA genes as recommended previously (Davis et al., 2003). We also provide data on two additional genomic loci for the designated "Ca. Phytoplasma pruni" reference strain PX11CT1C, as well as for other strains, since improved delineation of species should be achieved through analyses of more variable gene sequences (Lee et al., 2010; Martini et al., 2007). Availability of nucleotide sequences from multiple genetic loci, such as those encoding SecY and ribosomal proteins, especially for the reference strain of a 'Ca. Phytoplasma' (this paper) for the reference strain of 'Ca. Phytoplasma pruni' (data not shown). These examples may indicate that distinct species are signalled by a difference of at least one base in a minimum of two unique 16S rRNA gene regions designated for a described species; under this criterion, peach rosette (GenBank accession no. AF236121), little peach (AF236122), and peach red suture (AF236123) phytoplasmas would be considered (as additional strains of) 'Ca. Phytoplasma pruni' (Table 1). As more is learned about their biology and molecular genetics, it is possible that some 'Ca. Phytoplasma pruni'-related strains (as designated in this communication) could eventually be recognized as representing additional distinct species whose descriptions will incorporate unique regions of yet other species. Such progress seems likely to lead to emended descriptions of existing 'Ca. Phytoplasma' species that could include revisions of species-unique oligonucleotide sequences in 16S rRNA genes. Presumably, additional genes will become part of phytoplasma species descriptions, because evolutionary divergence probably proceeds largely through selection for ecological fitness determined by genes other than those encoding 16S rRNA (Davis & Sinclair, 1998).

In this communication, we propose recognition of a new species, 'Candidatus Phytoplasma pruni', principally on the basis of the gene encoding 16S rRNA. While current guidelines recommend analysis and description of only this single gene (from just one rRNA operon) for delineation of such a unique 'Candidatus Phytoplasma' species (IRPCM, 2004), we include nucleotide sequences for both sequence-heterogeneous 16S rRNA genes as recommended previously (Davis et al., 2003). We also provide data on two additional genomic loci for the designated 'Ca. Phytoplasma pruni' reference strain PX11CT1C, as well as for other strains, since improved delineation of species should be achieved through analyses of more variable gene sequences (Lee et al., 2010; Martini et al., 2007). Availability of nucleotide sequences from multiple genetic loci, such as those encoding SecY and ribosomal proteins, especially for the reference strain of a 'Ca. Phytoplasma'
species, should aid future efforts to assess whether various strain lineages within group 16SrIII may represent additional species. We propose that secY, rp, and other genetic loci (such as map, tuf, groEL, dnaK, gcp, rpoB, adhesion genes, and immunodominant protein genes) from the reference strain of a species, and where possible oligonucleotide sequences of their unique regions that distinguish species within a given 16Sr group, be incorporated
in emended descriptions and as part of future descriptions of ‘Candidatus Phytoplasma’ species.

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References


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