# Title: **'*Candidatus* Phytoplasma platensis', a novel taxon associated with daisy (*Bellis perennis*) virescence and related diseases in South America**

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**running title**: "*Candidatus* Phytoplasma platensis"

GenBank [/EMBL/DDBJ] accession numbers of gene sequence from representative strain ('Ca. Phytoplasma platensis' BellVir) are: MK135798, KC412019; MK140657, MG435348, MG435349 (16S rRNA, rplV-rps3, secA, imp and idpA respectively).

**Abstract**

Bellis perennis virescence (BellVir) phytoplasma affects ornamental daisies in Argentina. It has been previously classified within the X-disease group, subgroup III-J, which is one of the most important and widely distributed in South America, affecting diverse plant hosts. In this study, we compared 16S rRNA, ribosomal proteins rpIV and rps3, secA and immunodominant proteins imp and idpA genes of BellVir phytoplasma with previously described '*Candidatus* Phytoplasma' species. The 16S rRNA gene of strain BellVir shared less than 97.5% with all previously described '*Ca*. Phytoplasma' taxa except for '*Ca*. Phytoplasma pruni'. According to the recommended rules for the description of novel taxa within '*Ca*. Phytoplasma', it should be considered as '*Ca*. P. pruni'-related strain. However, multilocus analysis showed further molecular diversity that distinguished BellVir phytoplasma from '*Ca*. Phytoplasma pruni'. Besides, BellVir phytoplasma and 16SrIII-J related strains have a geographical distribution restricted to South America, where '*Ca*. P.pruni' has not been detected. Two insect vectors have been reported to transmit 16SrIII-J phytoplasmas, which have not been found to transmit '*Ca*. Phytoplasma pruni'. Having a wide host range, they have not been detected in *Prunus persica*. Therefore, based on multilocus sequence analyses, specific vector transmission and geographical distribution, we propose the recognition of the novel phytoplasma species '*Ca*. Phytoplasma platensis', within the X-disease clade, with Bellis perennis virescence phytoplasma as the reference strain.

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The provisional genus '*Candidatus* Phytoplasma', formally described in 2004, includes plant pathogenic non-helical *Mollicutes* that inhabit plant phloem and insects [1]. Phytoplasmas have been reported worldwide causing diseases in more than 1000 plant species [2]. In plant hosts they colonize mainly sieve tube elements but can reach

parenchymatic companion cells. They are transmitted among plant hosts by sap-sucking insects, such as leafhoppers and psyllids, in a persistent manner and could be detected in the insects' gut, hemolymph, salivary glands and other organs [3]. Symptomatic plants can show stunting, die back, abnormal shoot proliferation (witches' broom), leaf yellowing or reddening, reduced leaf size and deformation. Other symptoms include flower sterility, phyllody and virescence [4, 5].

The provisional status of *Candidatus* responds to the difficulty of obtaining pure cultures, and consequently phenotypic description. Instead, conserved gene sequence analysis has been used for the detection and classification of phytoplasma strains. Based on 16S rRNA gene sequence and RFLP analysis phytoplasmas have been classified into 16Sr groups and subgroups [6, 7, 8]. So far, more than thirty groups have been described, most of which host at least one '*Ca*. Phytoplasma species' [9, 10]. Further distinction can be achieved by including biological features such as plant hosts, symptoms, insect vectors, geographical distribution, and multilocus sequence analysis [11–14]. According to the recommended rules for the description of novel taxa within '*Ca*. Phytoplasma', a new species should refer to a unique 16S rRNA gene sequence with ≤97.5% similarity with a previously described '*Ca*. Phytoplasma species'. However, a strain that has higher sequence similarity could be described as a new species if it has clearly different biological characteristics including host range and vector transmission, and significative molecular diversity [1].

*Bellis perennis* virescence phytoplasma (BellVir) (Figure S1) was first reported in 2013 affecting ornamental daisies in Argentina. Based on 16S rRNA and ribosomal protein genes analyses it has been classified within group 16SrIII or X-disease group, subgroup 16SrIII-J [15]. Chayote witches' broom (ChWBIII) phytoplasma was the first 16SrIII-J strain detected in Brazil [16]. After that, subgroup 16SrIII-J phytoplasmas have been reported in various cultivated, domesticated and wild plant hosts in South America, mainly Argentina, Southern Brazil and Chile [15, 17–21]. Infected plant hosts

showed diverse symptoms including leaf size and general growth reduction, internode shortening and shoot proliferation, color changing, phyllody and virescence. The X-disease or 16SrIII is one of the most numerous and diverse groups, composed by more than 25 subgroups. Up to now, one '*Ca*. Phytoplasma' species has been described ('*Ca*. Phytoplasma pruni') that includes strains of group 16SrIII, with Peach X-disease phytoplasma from subgroup 16SrIII-A as reference strain (PX11CT1, JQ044392). Phytoplasmas from other 16SrIII subgroups have been considered as related strains since they show differences in the oligonucleotide unique regions of the rRNA gene [22], However, the high diversity within 16SrIII group led the authors to suggest that the analyzed 16SrIII group phytoplasma lineages might represent at least two, or even three different phytoplasma taxa..

*Bellis perennis* phytoplasma (BellVir, 16SrIII-J) [15] was selected as reference strain for molecular analyses. The phytoplasma was first transmitted from infected daisies to healthy periwinkle (*Catharanthus roseus* (L.) G. Don.) using *Cuscuta subinclusa*. Once the infection was established in periwinkle, the strain was perpetuated by periodical grafting. Typical symptoms of phyllody, virescence, leaf size reduction and yellowing were observed in infected plants 3 to 6 months after grafting (Figure S1). For PCR amplifications, genomic DNA was extracted from BellVir infected periwinkle leaves and petioles by the CTAB technique [23]. Phytoplasma 16S rRNA gene was amplified by PCR using primers P1/P7 [24] according to previously described protocol [15]. Partial secA gene amplification (~0.80kb) was performed using SecAfor1/SecArev3 primer pair following the conditions proposed by [25]. For immunodominant proteins, we designed specific primers based on the genomic information available in public database. So far, only one draft genome has been described for subgroup 16SrIII-J [26] which belongs to Vc33 phytoplasma isolate from periwinkle. Annotation pipeline  led us to identify imp and idpA gene sequences [27]. Two new sets of primers impXd-Fw1/impXd-Rv1 and idpAXd-Fw1/idpA-Rv1 were

designed in order to amplify by PCR a genomic fragment containing the complete sequence of imp and idpA genes, respectively (Table S3, Supplementary material). The amplicons were purified and cloned as described previously [28]. Three clones for each isolate were bidirectionally sequenced using an automated DNA Sanger sequencer (Unidad Genómica, Instituto de Biotecnología-Instituto Nacional de Tecnología Agropecuaria, Argentina). Final consensus sequences (3X coverage) were assembled using the Geneious R10 software and deposited in the GenBank nucleotide database. The phylogenetic reconstruction for each gene was performed using Maximum Likelihood method from the MEGA 6 software package [29].

**BellVir represents a novel taxon for the provisional genus 'Candidatus Phytoplasma'**

The signature sequence (5'-CAAGAYBATKATGTKTAGCYGGDCT-3') characteristic of the provisional genus '*Candidatus* Phytoplasma' is contained in BellVir´s 16S rRNA gene (5'-CAAGACTATGATGTGTAGCTGGACT-3') (263-287). The 16S rRNA gene (MK135798) of strain BellVir shared less than 97.5% with corresponding fragments of the 16S rRNA genes from all previously described '*Ca*. Phytoplasma' taxa except for '*Ca*. Phytoplasma pruni', with 98.79-98.87% nucleotide sequence identity (Table 1). However, multilocus analysis showed further molecular diversity that distinguished BellVir phytoplasma from '*Ca*. Phytoplasma pruni'. Besides, BellVir phytoplasma and 16SrIII-J related strains have a geographical distribution restricted to southern South America and have particular biological characteristics. Having a wide host range that includes *Bellis perennis* (used as reference strain), *Allium sativum*, *Cucurbita maxima*, *Coffea arabica*, *Solanum lycopersicum*, *S. melongea*, *Helianthus annus*, *Sechium edule*, *Brassica oleracea*, *Beta vulgaris*, *Fragaria* x *annanasa*, *Lactuca sativa*, *Manihot sculenta* and *Prunus avium* (cherry) among others[16, 20, 29–31, 33], phytoplasmas from this subgroup have not been detected in *Prunus persica* (peach). As regards vector transmission, two insect vectors

have been reported to transmit 16SrIII-J phytoplasmas, *Paratanus exitiosus* and *Bergallia valdiviana*, which have not been found to transmit '*Ca*. Phytoplasma pruni' [19, 34]. '*Ca*. Phytoplasma pruni' has not been detected until now in South American countries and, if 16SrIII-J phytoplasmas were considered related to it, attempts to regulate the pathogen introduction into these countries would be very difficult to accomplish.

The phylogenetic tree based on 16S rDNA sequences of BellVir phytoplasma and known '*Ca*. phytoplasma species' showed that BellVir clustered with '*Ca*. Phytoplasma pruni' but separated into an independent branch within the cluster (Figure 1). A broader phylogenetic analysis showed that '*Ca*. Phytoplasma platensis' and '*Ca*. Phytoplasma pruni' strains conform two well defined clades (Figure S2, supplementary material). Previous works had shown the same topology, separating 16SrIII-J phytoplasmas from other 16SrIII subgroups [15, 18, 21]. When the unique signature regions that distinguish '*Ca*. Phytoplasma pruni' where examined in BellVir's 16S rRNA gene sequence, 7 out of the 13 unique regions showed at least one nucleotide difference between them (Figure S3, supplementary material).

**Multilocus sequence analyses differentiate '*Candidatus* Phytyoplasma platensis' from '*Candidatus* Phytoplasma pruni'**

Other genomic regions were examined to determine molecular diversity of BellVir comparing with previously described '*Ca*. Phytoplasma pruni'. Sequences of ribosomal protein genes rpIV and rps3 of BellVir showed >98.5% identity with related strains and 96.5% with '*Ca*. Phytoplasma pruni'. RFLP patterns generated by restriction enzymes *Alu*I and *Dra*I distinguished BellVir from '*Ca*. Phytoplasma pruni' [15]. The phylogenetic tree showed that BellVir and related phytoplasmas integrate a cluster separated from '*Ca*. Phytoplasma pruni' and related strains (Figure 2). Multiple alignment of ribosomal proteins gene sequences revealed the presence of 17 SNPs

156 that distinguish '*Ca*. Phytoplasma platensis' strains from the 'Ca. Phytoplasma pruni'
157 strains (Table S2, supplementary material). Similar results were obtained when secA
158 gene was analyzed, and the amino acid sequence corresponding to BellVir secA
159 protein (MK140657) showed 99.4% identity with Vac33 strain (LLKK01000003) while
160 both of them shared a maximum identity of 96.6% with '*Ca*. Phytoplasma pruni' strains.
161 The resulting tree had the same topology as the generated by 16S rRNA and ribosomal
162 protein genes, supporting the separation of BellVir phytoplasma (Figure 3).
163 Immunodominant proteins would not be correlated with that of 16Sr DNA; however, imp
164 is a gene well conserved over a wide range of phytoplasmas and can reflect in
165 between phytoplasma differences regarding host range and vector transmission [35].
166 Phytoplasmas immunodominant proteins have been classified into three distinct types:
167 (i) immunodominant membrane protein (Imp); (ii) immunodominant membrane protein
168 A (IdpA); and (iii) antigenic membrane protein (Amp) [36]. BellVir phytoplasma has the
169 same type of immunodominant membrane proteins as all 16SrIII-group phytoplasmas
170 since both imp and idpA genes could be amplified [37]. BellVir imp amino acid
171 sequence had 97.7% identity with 16SrIII-J phytoplasma Vac33, and 58.3-61% identity
172 with '*Ca*. Phytoplasma pruni' related strains, which showed 97.7-79.6% identity among
173 them. The phylogenetic tree constructed with imp aa sequences of 16SrIII
174 phytoplasmas clearly separated BellVir from '*Ca*. Phytoplasma pruni' and related
175 strains (Figure S4, supplementary material). Similar situation arise within the idpA
176 protein, since the highest identity occurs with Vac33 phytoplasma (87.4%) and 66.26-
177 69.51% identity *Ca*. Phytoplasma pruni' related strains, which showed 99.65-63.61%
178 identity among them. The topology of the phylogenetic tree constructed with idpA aa
179 sequences resembled those of imp, and showed once again a clear separation of '*Ca*.
180 Phytoplasma platensis' and '*Ca*. Phytoplasma pruni' clades.

181 Based on multilocus sequence analyses, specific vector transmission and
182 geographical distribution, we propose the recognition of the new phytoplasma species

183 '*Ca*. Phytoplasma platensis', within the X-disease clade. The continuous advance in the
184 field of genomics and the fundamental biology of phytoplasmas will allow us to describe
185 new phytoplasma species.

186 **Description of '*Candidatus* Phytoplasma platensis'**

187 '*Candidatus* Phytoplasma platensis' (pla. ten'. sis. L. masc. adj. referring to Río de la
188 Plata, a river representative of Argentina and southern South America, where the
189 reference strain was identified).

190 [(Mollicutes) NC; NA; O, wall less; NAS (GenBank accession number XXXX),
191 oligonucleotide sequences of unique regions of the 16S rRNA gene; 5'-617-
192 CTATAGAAACTGTTTTACTAGAGTGAGTTAGAGGCAAG-654-3' (*Bellis perennis*,
193 phloem); M].

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197 **Conflicts of interest**

198 The authors declare that there are no conflicts of interest

199 **Ethical statement**

200 No humans or animals were subjects in this work.

201

202 **References**

203 1. **IRPCM Phytoplasma/Spiroplasma Working Team–Phytoplasma Taxonomy**
204 **Group.** '*Candidatus* Phytoplasma', a taxon for the wallless, non-helical
205 prokaryotes that colonize plant phloem and insects. Int J Syst Evol Microbiol

2004;54:1243–1255.

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. **Lee I-M, Davis RE, Gundersen-Rindal DE**. PHYTOPLASMA□: Phytopathogenic Mollicutes. *Annu Rev Microbiol* 2000;54:221–255.

5. **Bertaccini A**. Phytoplasmas: diversity, taxonomy, and epidemiology. *Front Biosci* 2007;12:673.

6. **Gundersen D., Lee I.** Ultrasensitive by nested-PCR assays detection of phytoplasmas using two universal primer pairs. *Mediterr Phytopathol* 1996;35:144–151.

7. **Lee I-M, Hammond R, Davis RE, Gundersen DE .**Universal amplification and analysis of pathogen 16S rDNA for classification and identification of MLOs. *Phytopathol* **83**, 834–842.

8. **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 . *Int J Syst Bacteriol* 1998;1153–1169.

9. **Miyazaki A, Shigaki T, Koinuma H, Iwabuchi N, Rauka GB, *et al.*** Candidatus phytoplasma noviguineense, a novel taxon associated with bogia coconut syndrome and banana wilt disease on the island of new Guinea. *Int J Syst Evol Microbiol* 2018;68:170–175.

10. **Zhao Y, Wei W, Lee IM, 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.

11. **Lee IM, Gundersen-Rindal DE, Davis RE, Bottner KD, Marcone C, *et al.*** 'Candidatus Phytoplasma asteris', a novel phytoplasma taxon associated with aster yellows and related diseases. *Int J Syst Evol Microbiol* 2004;54:1037–1048.

12. **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.

13. **Hodgetts J, Boonham N, Mumford R, Dickinson M**. Panel of 23S rRNA gene-based real-time PCR assays for improved universal and group-specific detection of phytoplasmas. *Appl Environ Microbiol* 2009;75:2945–2950.

14. **Lee IM, Bottner-Parker KD, Zhao Y, Davis RE, Harrison NA**. Phylogenetic analysis and delineation of phytoplasmas based on secY gene sequences. *Int J Syst Evol Microbiol* 2010;60:2887–2897.

15. **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.

16. **Montano HG, Davis RE, Dally EL, Pimentel JP, Brioso PST**. Identification and phylogenetic analysis of a new phytoplasma from diseased chayote in Brazil. *Plant Dis* 2000;84:429–436.

17. **Mello AP de OA, Eckstein B, Flores D, Kreyci PF, Bedendo IP**. Identification by computer-simulated RFLP of phytoplasmas associated with eggplant giant calyx representative of two subgroups, a lineage of 16SrIII-J and the new

subgroup 16SrIII-U. *Int J Syst Evol Microbiol* 2011;61:1454–1461.

18. **Guzmán FA, Giolitti F, Fernández F, Nome C, Lenardon S, *et al.*** Identification and molecular characterization of a phytoplasma associated with sunflower in Argentina. *Eur J Plant Pathol* 2014;138:679–683.

19. **Fiore N, Longone V, Gonzalez X, Zamorano A, Pino AM, *et al.*** Transmission of 16SrIII-J phytoplasma by Paratanus exitiosus leafhopper in grapevine. *Phytopathogenic mollicutes* 2015;5:43–44.

20. **Fugita JMS, Pereira TBC, Banzato TC, Kitajima EW, Souto ER, *et al.*** Occurrence of a subgroup 16SrIII-J phytoplasma in non-symptomatic Brachiaria decumbens cultivated in a grazing area. *Trop Plant Pathol* 2017;42:500–503.

21. **Fernández F, Uset A, Baumgratz G, Conci L**. Detection and identification of a 16SrIII-J phytoplasma affecting cassava ( Manihot esculenta Crantz ) in Argentina. 2018;1–5.

22. **Davis RE, Zhao Y, Dally EL, Lee IM, Jomantiene R, *et al.*** '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. *Int J Syst Evol Microbiol* 2013;63:766–776.

23. **Doyle J**. Isolation of plant DNA from fresh tissue. *Focus (Madison)* 1990;12:13–15.

24. **Deng S, Hiruki C**. Amplification of 16S rRNA genes from culturable and unculturable Mollicutes. *J Microbiol Methods* 1991;14:53–61.

25. **Hodgetts J, Boonham N, Mumford R, Harrison N, Dickinson M**. Phytoplasma phylogenetics based on analysis of secA and 23S rRNA gene sequences for improved resolution of candidate species of 'Candidatus Phytoplasma'. *Int J Syst Evol Microbiol* 2008;58:1826–1837.

280 26. **Zamorano A, Fiore N**. Draft Genome Sequence of 16SrIII-J Phytoplasma, a
281 Plant Pathogenic Bacterium with a Broad Spectrum of Hosts. *Genome Announc*
282 2016;4:e00602-16.

283 27. F**ernandez FD, Debat HJ, Conci LR.** Molecular characterization of effector protein
284 SAP54 in Bellis Virescence Phytoplasma (16SrIII-J). bioRxiv, doi:
285 https://doi.org/10.1101/411140

286 28. **Fernández FD, Galdeano E, Kornowski MV, Arneodo JD, Conci LR**.
287 Description of 'Candidatus Phytoplasma meliae', a phytoplasma associated with
288 Chinaberry (Melia azedarach L.) yellowing in South America. *Int J Syst Evol*
289 *Microbiol* 2016;66:5244–5251.

290 29. **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S**. MEGA6: Molecular
291 evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725–2729.

292 30. **Galdeano E, Torres LE, Meneguzzi N, Guzmán F, Gomez GG, *et al.***
293 Molecular characterization of 16S ribosomal DNA and phylogenetic analysis of
294 two X-disease group phytoplasmas affecting China-tree (Melia azedarach L.)
295 and garlic (Allium sativum L.) in Argentina. *J Phytopathol* 2004;152:174–181.

296 31. **Montano HG, Cunha JO, Pimentel JP**. Phytoplasmas in Brazil: An update. *Bull*
297 *Insectology* 2011;64:251–252.

298 32. **Quiroga N, Bustamante M, Gamboa C, Molina J, Zamorano A, *et al.*** 16SrIII-J
299 phytoplasmas infecting lettuce and Swiss chard crops in Chile. *Phytopathogenic*
300 *Mollicutes* 2017;7:91.

301 33. **González F, Zamorano A, Pino AM, Paltrinieri S, Bertaccini A, *et al.***
302 Identification of phytoplasma belonging to X-disease group in cherry in Chile.
303 *Bull Insectology* 2011;64:235–236.

304 34. **Quiroga N, Zamorano A, Pino AM, Fiore N**. Transmission of 16SrIII-J

Phytoplasmas to Grapevine by Bergallia valdiviana Leafhopper. *Phytopathogenic mollicutes* 2015;10:99–100.

35. **Konnerth A, Krezal G, Boonrod K**. Immunodominant membrane proteins of phytoplasmas. *Microbiol (United Kingdom)* 2016;162:1267–1273.

36. **Kakizawa S, Oshima K, Jung H, Suzuki S, Nishigawa H, *et al.*** Positive Selection Acting on a Surface Membrane Protein of the Plant-Pathogenic Phytoplasmas Positive Selection Acting on a Surface Membrane Protein of the Plant-Pathogenic Phytoplasmas †. *Society* 2006;188:3424–3428.

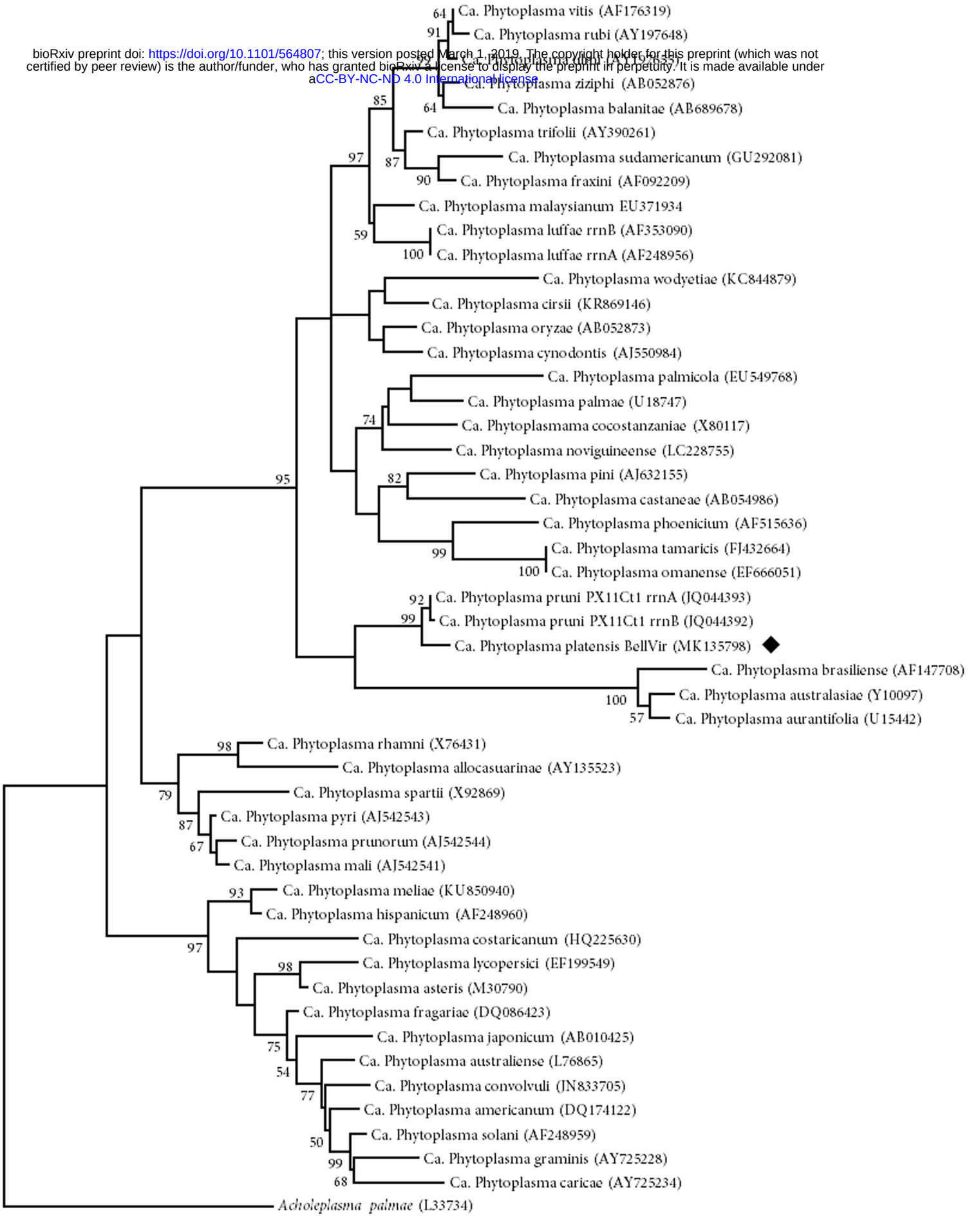
37. **Neriya Y, Sugawara K, Maejima K, Hashimoto M, Komatsu K, *et al.*** Cloning, expression analysis, and sequence diversity of genes encoding two different immunodominant membrane proteins in poinsettia branch-inducing phytoplasma (PoiBI). *FEMS Microbiol Lett* 2011;324:38–47.

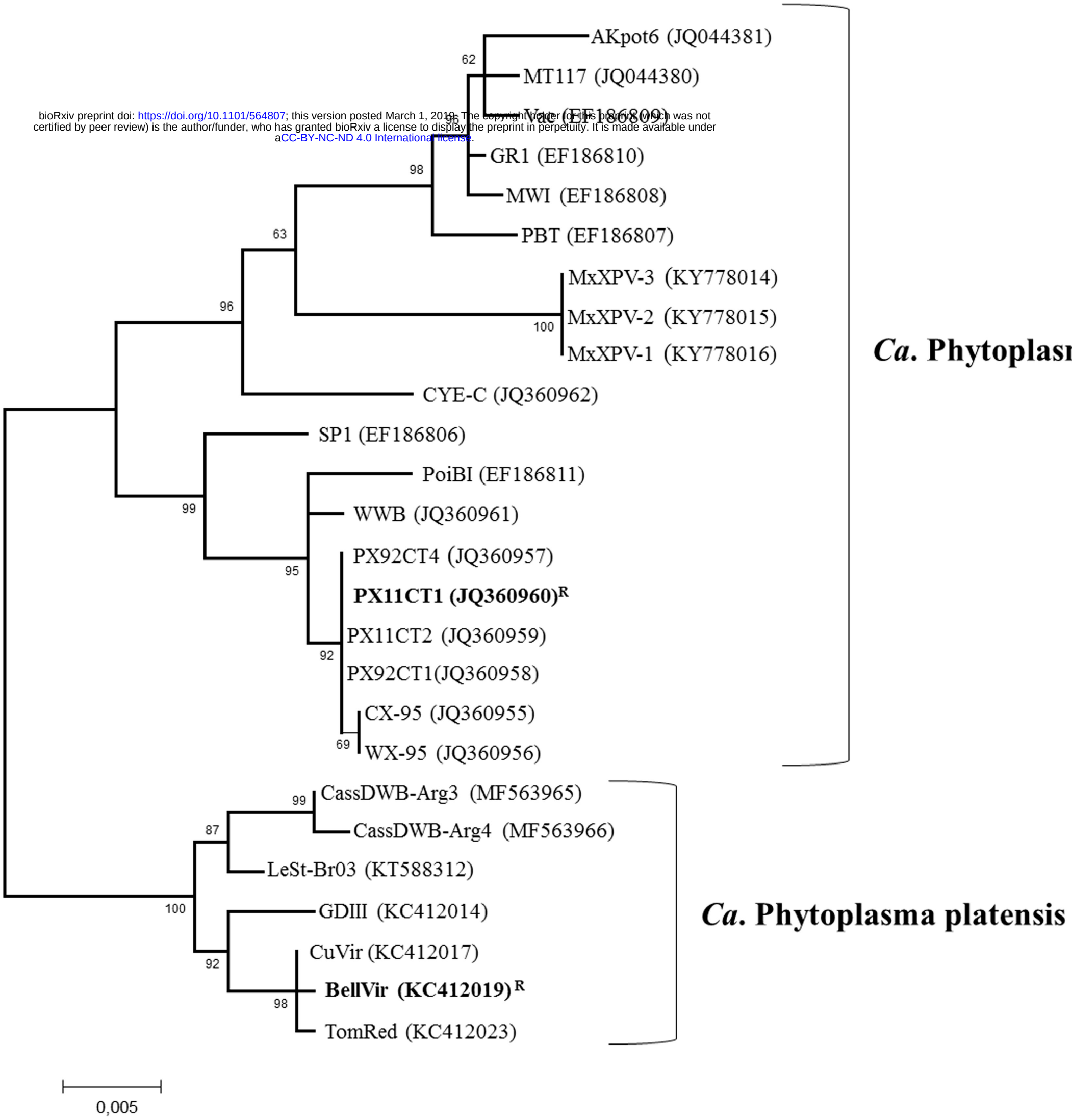
## Figure Legends

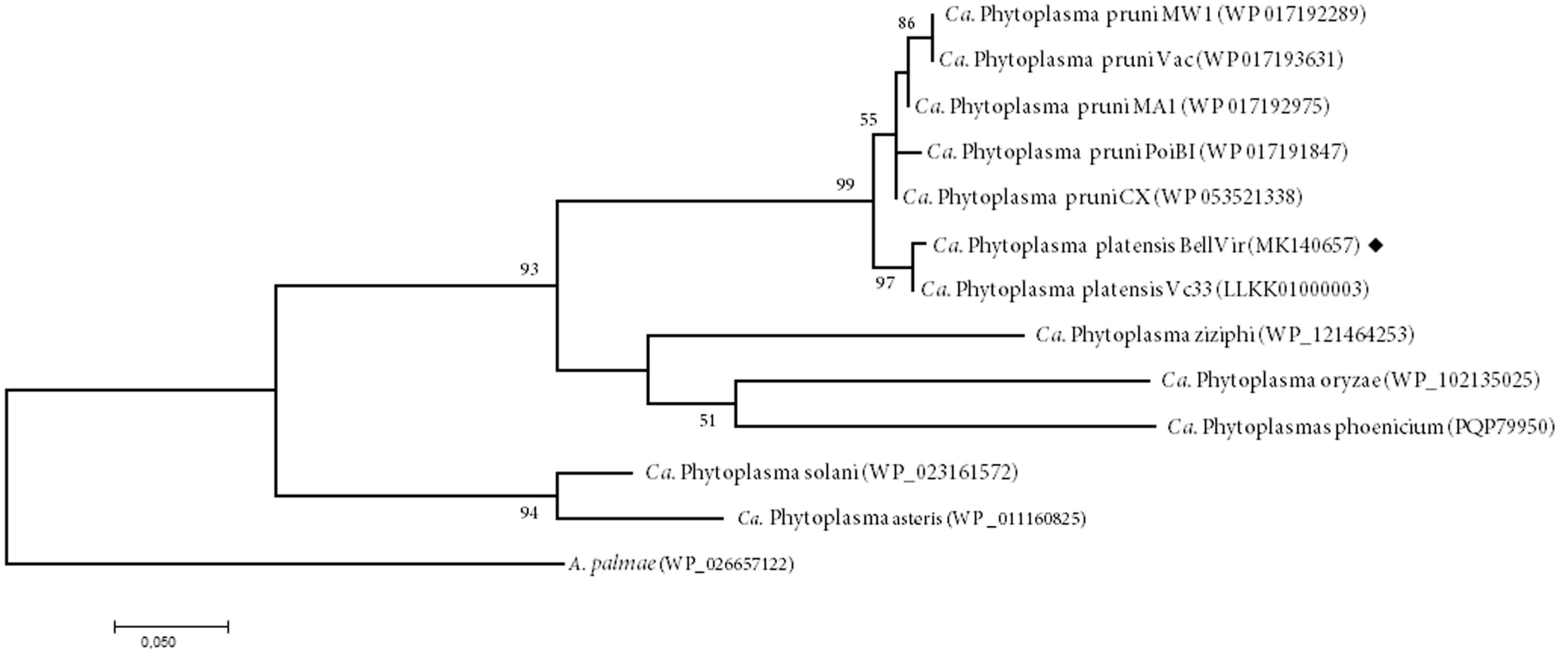
**Figure 1:** Phylogenetic tree inferred from 16S rRNA gene sequence analysis using the Maximum Likelihood method implemented in the Molecular Evolutionary Genetics Analysis program (MEGA 6). *Acholeplasma palmae* was used as outgroup. The numbers on the branches are bootstrap (confidence) values (expressed as percentage of 1000 replicates). GenBank accession number for each taxon is given between parentheses. The final tree includes reference strain of 48 previously described or incidentally cited as 'Ca. Phytoplasma species' and 'Ca. Phytoplasma platensis' (marked with black diamond). Bar, number of nucleotide substitutions per site.

**Figure 2:** Phylogenetic tree inferred from rplV and rps3 genes sequence analysis using the Maximum Likelihood method implemented in the Molecular Evolutionary Genetics Analysis program (MEGA 6). The numbers on the branches are bootstrap (confidence) values (expressed as percentage of 1000 replicates). GenBank accession number for each taxon is given between parentheses. R: reference strains. Bar, number of nucleotide substitutions per site.

**Figure 3:** Phylogenetic tree inferred from secA aa sequence analysis using the Maximum Likelihood method implemented in the Molecular Evolutionary Genetics Analysis program (MEGA 6). The numbers on the branches are bootstrap (confidence) values (expressed as percentage of 1000 replicates). GenBank accession number for each taxon is given between parentheses. Sequence of 'Ca. platensis' obtained in this work is marked with black diamond. Bar, number of nucleotide substitutions per site.



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Ca. Phytoplasma pruni MA1 (WP 017192975)
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