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REVISED CRITERIA FOR THE CLASSIFICATION OF '*CANDIDATUS* PHYTOPLASMA': IMPLICATIONS FOR EPIDEMIOLOGY AND ECOLOGICAL STUDIES

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ABSTRACT

The genus '*Candidatus* Phytoplasma' was presented to group the cell-wall-less microorganisms living in the plant phloem tissue and animal vector tissues. Apart from the Viruses, it is important to observe that these microorganisms have not been well elucidated in terms of molecular or biochemical properties. This provisional classification is especially important in epidemiological and ecological investigation, oriented at the modulation of dangerous phytoplasma-associated plant diseases all over the world. Owing to discovery of more diversity within the genus '*Ca.* Phytoplasma', the above-mentioned criteria have been deemed relevant. The criteria now state that any '*Ca.* Phytoplasma' species strain with more than 98.65% sequence identity in both its total or near total 16SrRNA gene sequence and with twofold sequence coverage should be compared to the standard strain of the species. Any two strains have the same percentage (< 1%) in their DNA sequence identity of strains belonging to the same '*Ca.* Phytoplasma' species. The reference strains are retained in the updated criteria but in addition to the reference strains there are proposed complementary strains. This alternative approach is particularly advisable where only partial 16SrRNA gene and several other genes have been sequenced or the original strain is no longer amenable for the additional molecular analysis. Lists of the '*Ca.* Phytoplasma' species and the strains used in this study as reference strains are included below. In order to define new '*Ca.* Phytoplasma' species having 16SrRNA gene sequence similarity of 98.65% or higher, an average nucleotide identity of 95% genome wide is suggested. If full genomic sequences are not attainable, two conservative housekeeping genes commonly present in all organisms could be utilised.

This paper is a description of two of the 49 officially recognized bacterial species belonging to the '*Candidatus* Phytoplasma' genus, more specifically '*Ca.* P. cocostanzaniae' and '*Ca.* P. palmae'.

***Keywords*: *Candidatus* Phytoplasma, insect vector tissues, genome, nucleotide.**

Introduction

The genus '*Candidatus* Phytoplasma' (IRPCM) was created for classification of non-helical, cell wall-less bacteria which reside in plant phloem tissue and tissues of their insect vectors. These bacteria come under the Mollicutes class and they are implicated in more than 1000 plant diseases worldwide. Phytoplasma cannot be grown in a pure culture (Contaldo *et al.*, 2016) and many of these pathogens have not yet been successfully cultured in the laboratory and thus the Koch postulates which eliminate phytoplasma's pathogen status have not been met. Phytoplasma diseases are insect borne and reported from different countries of the world. Since disease detection is one of the environmental factors that can help in understanding the occurrence of these diseases, the assessment of phytoplasma species, in geographically regions, is useful for controlling and preventing the diseases (Montano *et. al.*, 2024). Phytoplasmas are associated with diseases in thousands of economically important crops such as carrot as stated by Toth R. *et. al*, 2024. They are still regularly classified despite the fact they are still little known about their biological distinctive features. Therefore, a provisional genus designation was proposed for investigation of their epidemiology and genotypes, although some of them has new molecular profiles (Namba, 2019). An increasing number of taxa has been recognized and characterized and taken their features might overlap, the definition of a '*Ca.* Phytoplasma' species must be modified and refined.

Threshold for Defining '*Candidatus* Phytoplasma' Species Based on the 16S rRNA Gene

Earlier, it was proposed that '*Ca.* Phytoplasma' species should share < 3% difference in their 16SrRNA gene sequence for them to be classified as different species; however, this was changed in 2004 to include the correct 16SrRNA gene length for this classification (Yarza *et al.,* 2014). For new '*Ca.* Phytoplasma' species description, three genetic distances (97.50%, 98.00%, and 98.65%) have been tested on existing 16SrRNA gene sequences, based on the walled bacteria criteria (Kim M. *et al.,* 2014). This comparison (Tables 1 and S1, Table S1) showed that, for example, using a higher cut-off of 98.65% would increase the number of '*Ca.* Phytoplasma' species: up to splitting of presently included species, such as '*Ca.* P. phoenicium', '*Ca.* P. pruni', but would decrease the number of misclassified strains. Aleration of existing annotation as per the pointers of this evaluation, of '*Ca.* Phytoplasma' species suggest that 13 species have changes in the number of strains formerly assigned and, which must be re-analyzed to ensure that they are properly re-grouped in a new taxonomy based on this study.

In bacterial taxonomy, Comparison of the genome is well done by the average nucleotide identity abbreviated as ANI which is a good and well reliable tool for genome comparison (Goris *et al.,* 2007; Richter *et al.,* 2009). For two species, an ANI of 95 to 96% was suggested (Yarza *et al.,* Kim *et al.,* 2014). So, differentiation should be carried out based on only 1.5 Kb long DNA sequence in the middle of 16SrRNA gene, most probably containing about 95% of the whole gene without both regions which codes for primers. This sequence should be derived from both strands obtained via Sanger sequencing with at least twofold coverage from three independent biological samples or three different locations where a single phytoplasma infection was identified. Primer pairs that amplify the entire 16SrRNA gene include P1/P7, P1/16S-SR, or P1A/16S-SR (Bertaccini *et al.,* 2019). Strains sharing more than 98.65% sequence identity with the reference strain are considered members of the respective '*Ca.* Phytoplasma' species. Strains with 98.65% identity to other strains of the same '*Ca.* Phytoplasma' species should be regarded as related to that species. Since some '*Ca.* Phytoplasma' species only have partial 16S rRNA gene sequences available for the originally designated reference strains, additional complementary strains with longer 16SrRNA sequences and a larger number of available housekeeping gene sequences are recommended (Martini *et al.,* 2019) (Tables 2 and 3).

Updates on '*Candidatus* Phytoplasma' Species

PaWB disease or Paulownia witches' broom disease is a destructive disease in East Asia that affects the growth of paulownia tree due to 16SrI-D subgroup phytoplasma. The PaWB phytoplasmas are mainly transmitted by insects belonging to the Pentatomidae (stink bugs), Miridae (mirid bugs) and Cicadellidae (leaf hoppers) (Zhang *et al.,* 2024). For 16SrRNA gene sequence similarity higher than 98.65% similarity of determined sequence to the closest described species, the new '*Ca.* Phytoplasma' species will be defined by the 98.65% cut-off with other species. Furthermore, at least two of such genes under the neutral selection, have to be presented, If two phytoplasmas have greater than 98.65% similarity in 16SrRNA gene sequence, but has other molecular differences when distinguished, then the reported biological properties can help in describing the species. However, when an insect vector is added to substantiate a '*Ca.* Phytoplasma' species, this vector must be confirmed by a biological approach and species authenticity must be confirmed based on 16SrRNA, conserved gene, or housekeeping gene sequences.

General Observations on Published '*Candidatus* Phytoplasma' Species

The '*Ca.* Phytoplasma' species described to date have been classified according to the accessibility of full-length 16SrRNA gene sequences and other conserved genes showing homologies, and the bacterial species are listed in alphabetical order. Table 2 provides a list of 14 '*Ca.* Phytoplasma' species and alternative reference strains for which complete and partial length of the 16SrRNA gene and other genes, namely tufB, secA, secY, rpIV-rpsC, and groEL , are available. Table 3 also contains '*Ca.* Phytoplasma' species and strains with the complete 16S rRNA gene sequences, other sequences of conserved or housekeeping genes. In the current taxonomy, there are thirteen recognized '*Ca.* Phytoplasma' species for which the only easily accessible unique gene sequence data are the full-length 16SrRNA gene sequence data.

Moreover, six others '*Ca.* Phytoplasma' species have their 16SrRNA gene sequences beginning from different nucleotide positions as compared with the complete sequence of '*Ca.* P. asteris'. For instance, sequences starting at nucleotide position six include: For instance, sequences starting at nucleotide position six include:

- HibWB26 (GenBank accession number AF147708) of the '*Ca.* P. brasiliense' strain (Montano *et al.,* 2001).
- category: '*Ca.* P. lycopersici' strain Santa Cruz (GenBank accession number EF199549) (Arocha *et al.,* 2007).
- '*Ca.* P. oryzae' Strain: RYD, (GenBank accession no: D12581) Ref: Jung *et al.,* 2003).
- '*Ca.* Here we reported an isolate, P. palmicola' strain LYDM-178 (GenBank accession number: KF751387) (Harrison *et al.,* 2022).
- '*Ca.* Host'P. sudamericanum' strain PassWB-Br3 (GenBank accession number GU292081) (Davis *et al.,* 2012).
- From the database the name of the isolated '*Ca.* P. tamaricis' strain is SCWB1 with the accession number FJ432664 (Zhao *et al.,* 2009).

In addition, it has to be pointed out that the genetic sequences of '*Ca.* P. balanitae' strain BltWB (Win *et al.,* 2013) GenBank accession number: AB689678) and of '*Ca.* P. spartii' strain SpaWB (Marcone *et al.,* 2004) (GenBank accession number: X92869) begin at different nucleotide.

Table 1 : Strain composition of '*Ca.* Phytoplasma' species at diverse 16SrRNA gene sequence identity thresholds Analysis performed with blastn (https://www.ncbi.nlm.nih.gov) with the following settings:

***Ca.* Phytoplasma' Species**	**Min/Max Sequence Identity (%) vs Reference Strain**	**No. of Member Strains**	**No. of Related Strains ≥97.5%**	**No. of Related Strains ≥98%**	**No. of Related Strains ≥98.65%**	**No. of Related Strains ≥98%**	**No. of Related Strains ≥98.65%**
'*Ca.* P. allocasuarinae'	98.52	1	1	0	0	0	1
'*Ca.* P. americanum'	99.67/99.87	4	4	4	0	0	0
'*Ca.* P. asteris'	97.77/100	374	372	366	2	8	0
'*Ca.* P. aurantifolia'	97.50/99.73	293	265	97	28	196	0
'Ca. P. australasia'	97.51/100	236	229	175	7	61	0
'Ca. P. australiense'	98.68/99.93	12	12	12	0	0	0
'*Ca.* P. balanitae'	99.41/99.80	13	13	13	0	0	0
'*Ca.* P. brasiliense'	98.73/99.93	10	10	10	0	0	0
'*Ca.* P. caricae'*	-	-	-	-	-	-	-
'*Ca.* P. castaneae'*	-	-	-	-	-	-	-
'*Ca.* P. cirsii'	99.93/100	2	2	2	0	0	0
'*Ca.* P. cocostanzaniae'	99.33/100	17	17	17	0	0	0
'*Ca.* P. convolvuli'	99.93/100	9	9	9	0	0	0
'*Ca.* P. costaricanum'	99.15/99.61	17	17	17	0	0	0
'*Ca.* P. cynodontis'	98.37/100	36	36	35	0	1	0
'*Ca.* P. dypsidis'	99.83/99.88	6	6	6	0	0	0
'*Ca.* P. fragariae'	97.67/99.93	15	14	10	1	5	0
'*Ca.* P. fraxini'	97.61/99.93	22	17	9	5	13	0
'*Ca.* P. graminis'	98.34/99.74	5	5	4	0	1	0
'*Ca.* P. hispanicum'	98.53/99.47	7	7	6	0	1	0
'*Ca.* P. japonicum'*	-	-	-	-	-	-	-
'*Ca.* P. luffae'	99.87/99.93	13	13	13	0	0	0
'*Ca.* P. lycopersici'*	-	-	-	-	-	-	-
'*Ca.* P. malaysianum'	99.54	1	1	1	0	0	0
'*Ca.* P. mali'	99.74/100	20	20	20	0	0	0
'*Ca.* P. meliae'	99.45/99.86	5	5	5	0	0	0
'*Ca.* P. noviguineense'	99.66/100	26	26	26	0	0	0
'*Ca.* P. omanense'	99.58	1	1	1	0	0	0
'*Ca.* P. oryzae'*	-	-	-	-	-	-	-
'*Ca.* P. palmae'	98.05/100	80	80	66	0	14	0
'*Ca.* P. palmicola'	99.28/100	24	24	24	0	0	0
'*Ca.* P. phoenicium'	97.54/99.93	78	73	51	5	27	0
'*Ca.* P. pini'	99.74/99.93	3	3	3	0	0	0
'*Ca.* P. pruni'	97.87/100	207	205	203	2	4	0

'*Ca.* P. prunorum'	99.50/100	35	35	35	0	0	0
'*Ca.* P. pyri'	99.14/100	34	34	34	0	0	0
'*Ca.* P. rhamni'	100	1	1	1	0	0	0
'*Ca.* P. rubi'	99.35/99.77	7	7	7	0	0	0
'*Ca.* P. sacchari'	98.68/99.93	30	30	30	0	0	0
'*Ca.* P. solani'	98.17/99.93	73	72	72	1	1	0
'*Ca.* P. spartii'*	-	-	-	-	-	-	-
'*Ca.* P. stylosanthis'	99.94	1	1	1	0	0	0
'*Ca.* P. sudamericanum'*	-	-	-	-	-	-	-
'*Ca.* P. tamaricis'*	-	-	-	-	-	-	-
'*Ca.* P. trifolii'	97.98/100	80	79	76	1	4	0
'*Ca.* P. tritici'	100	1	1	1	0	0	0
'Ca. P. ulmi'	99.53/99.93	14	14	14	0	0	0
'*Ca.* P. wodyetiae'	98.48	1	1	0	0	0	1
'*Ca.* P. ziziphi'	99.54/100	33	33	33	0	0	0

*Only one strain available for comparison.

**Bold indicates the number of '*Ca.* Phytoplasma' species in which reassignment is needed to follow the revised guidelines.

The sequence of '*Ca.* P. castaneae' strain CnWB (Jung *et al.,* 2002) (GenBank accession number AB054986) starts at nucleotide 10, for '*Ca.* P. dypsidis' strain RID7941 (Jones *et al.,* 2021) (GenBank accession number MT293886) at nucleotide 25; for '*Ca.* P. caricae' strain PAY (Arocha *et al.,* 2005) (GenBank accession number AY725234) and '*Ca.* P. graminis' strain SCYLP (Arocha *et al.,* 2005) (GenBank accession number AY725228) at nucleotide 28 and for '*Ca.* P. costaricanum' strain SoyST1c1 (Lee *et al.,* 2011) (GenBank accession number HQ225630) at nucleotide 31. The four following '*Ca.* Phytoplasma' species have their 16SrRNA gene sequences starting after nucleotide 100 and are considered too short according to the newly proposed guidelines. The '*Ca.* P. stylosanthis' strain VPRI 43683 (Jardim *et al.,* 2021) (GenBank accession number MT431550) sequence starts at nucleotide 169. However, its tufB, secA and rplV-rpsC gene sequences are available under GenBank accession numbers MT432813 (364 nt); MT432821 (291 nt); and MT461153 (1257 nt), respectively. The '*Ca.* P. omanense', strain IM-1 (Al-Saady *et al.,* 2008) (GenBank accession number EF666051) sequence starts at nucleotide 116, the '*Ca.* P. wodyetiae' strain FPYD Bangi-2 (Naderali *et al.,* 2017) (GenBank accession number KC844879) sequence spans nucleotides 149 and 1399, and the '*Ca.* P. allocasuarinae', strain AlloY (Marcone *et al.,* 2004) (GenBank accession number AY135523) sequence spans nucleotides 370 and 1527. These '*Ca.* Phytoplasma' species sequences must be completed for the same strain or for an alternative reference strain.

'*Candidatus* Phytoplasma Palmae' and '*Candidatus* Phytoplasma Cocostazaniae Description'

Following the previous (IRPCM) and revised guidelines described in this publication, the following two '*Ca.* Phytoplasma' species are described, including some of their epidemiological and phytopathological traits (Table 4). '*Ca.* P. cocostanzaniae' It includes 17 phytoplasma strains associated with coconut lethal yellowing disease in Africa, mainly distributed in Tanzania. The proposed reference strain is LD, associated with the Tanzanian coconut lethal disease (Schuiling *et al.,* 1981 & Tymon *et al.,* 1998). The 16SrRNA sequences of 17 strains are deposited, and strain Tanz08-05 (GenBank accession number GU952106) also comprises the spacer region (1718 nucleotides). Compared to the reference strains of other known or newly designated '*Ca.* Phytoplasma' species, the LD strain shares the highest 16SrRNA gene sequence identity (96.30%) with that of the newly proposed reference strain '*Ca.* P. palmae' (Table 4). Unique signature sequences (position related to the 16SrRNA gene sequence of the reference strain) were identified Bertaccini *et al.,* Int. J. Syst. Evol. Microbiol. 2022;72: 005353.

Table 2 : Fourteen '*Ca.* Phytoplasma' species with full 16SrRNA gene sequences and five other gene sequences available for both reference and alternative reference strains GenBank numbers, available acronyms of the phytoplasma strains and nucleotide length, span and, for the 16SrRNA gene, the starting nucleotide are reported.

'*Candidatus* Phytoplasma'	**16S rRNA (nt; start)**	**tufB (nt)**	**secA (nt)**	**secY (nt)**	**rplV-rpsC (nt)**	**groEL (nt)**
'*Ca.* P. asteris'	M30790 (1542; 1) strain OAY (=MIAY)	AP006628 (1534; 279394–280928 and 555984–557518) strain OY-M	AP006628 (1185; 305131–306315)	AP006628 (2507; 530192–532699)	AP006628 (1241; 252238–253479)	AP006628 (1131; 246003–247134)
'*Ca.* P. aurantifolia'	U15442 (1513; 18) strain WBDL	NZ_MWKN01000002 (1202; 5087–6289)	NZ_MWKN01000041 (2495; 8846–11341)	NZ_MWKN01000015 (1262; 11153–12415)	NZ_MWKN01000015 (1085; 4491–5576)	NZ_MWKN01000043 (1640; 14982–16622)
'*Ca.* P. australiense'	L76865 (1375; 156) strain AUSGY	JQ824254 (391)	AM422018 (1521; 682142–683674) strain CaPaus	AM422018 (1185; 656879–658063)	AM422018 (2499; 557807–560305)	AM422018 (1248; 573941–575188)
'*Ca.* P. mali'	AJ542541 (1784; 7) strain AP15	CU469464 (1522; 264144–265665 and 450385–451906) strain AT	CU469464 (1178; 474914–476092)	CU469464 (2393; 134124–131731)	CU469464 (1244; 433420–434664)	CU469464 (1244; 427172–428288)
'*Ca.* P. meliae'	KU850940 (1528; 6) strain ChTY-Mo3	KU850948 (681)	KU850944 (1259)	NZ_JAGVRH010000003 (1532; 33957–35489) strain StrPh-Cl	NZ_JAGVRH010000003 (1184; 59600–60784)	NZ_JAGVRH010000007 (2504; 3107–5611)
'*Ca.* P. pini'	AJ632155 (1537; 6) strain Pin127S	VIAE01000001 (1518; 19320–20838) strain MDPP	VIAE01000003 (1227; 13731–12505)	VIAE01000005 (2498; 22267–19769)	VIAE01000002 (1326; 25071–23746)	VIAE01000002 (1141; 31440–30300)
'*Ca.* P. phoenicium'	AF515636 (1502; 31) strain A4	KM275491 (1250) strain SA213	KM275492 (1185)	JPSQ01000034 (2415; 2–2416)	JPSQ01000002 (1311; 11880–13190)	JPSQ01000002 (1102; 18377–19478)
'*Ca.* P. pruni'	JQ044397 (1527; 14) strain CX-95	LHCF01000002 (1184; 8092–9276)	LHCF01000002 (2507; 49619–52126)	JQ268249 (1263)	JQ360955 (1239)	LHCF01000008 (164; 22632–22796)
'*Ca.* P. pyri'	AJ542543 (1516; 7) strain PD1	MZ507700 (1179)	MZ507699 (2394)	GU004363 (269-1489)	EF193370 (1117)	MZ507698 (1614)
'*Ca.* P. sacchari'	MN889545 (1516; 7) strain SCGS	VWXM01000002 (1220; 9425–10645)	VWXM01000004 (2510; 3173–5683)	VWXM01000002 (1276; 26714–27990)	VWXM01000002 (1044; 20387–21431)	VWXM01000012 (1105; 1–1105)

'*Ca.* P. solani'	AF248959 (1527; 6) strain STOL	JQ797670 (946)	JQ797668 (1224)	JQ797662 (1093)	JQ730740 (1491; 44) strain 284/09	FO393427 (1185; 515449–516633)
'*Ca.* P. tritici'	DQ078304 (1432; 53) strain WBD	AVAO01000003 (1184; c230832-232016)	AVAO01000003 (2501; 112769–115276)	AVAO01000003 (1241; c282126-283367)	AVAO01000003 (691; 289222–289913)	AVAO01000001 (1610; 47299–48909)
'*Ca.* P. ulmi'	AY197655 (1527; 6) strain EY1	FN561879 (925)	KJ462034 (559)	AY197690 (1433)	AY197675 (1199)	OU413475 (1527; 65) strain ULW
'*Ca.* P. ziziphi'	AB052876 (1367; 10) strain JWB-G1	CP025121 (1531; 423854–425385 and 597926–599457) strain JWB-nky	CP025121 (1163; 478755–479918)	CP025121 (2513; 365998–368511)	CP025121 (1247; 4888–6135)	

Note:
nt stands for nucleotide.
start denotes the starting nucleotide position of the sequence.
The data is based on the table from the reference "Bertaccini *et al.,* Int. J. Syst. Evol. Microbiol. 2022;72: 005353".

Table 3 : Sixteen '*Ca.* Phytoplasma' species with full 16SrRNA genes and other available gene sequences for reference or alternative reference strains GenBank numbers, available acronyms of the phytoplasma strains and nucleotide length, span and, for the 16SrRNA gene, the starting nucleotide are reported.

'*Candidatus* Phytoplasma'	**16S rRNA (nt; start)**	**tufB (nt)**	**secA (nt)**	**secY (nt)**	**rplV-rpsC (nt)**	**groEL (nt)**
'*Ca.* P. americanum'	DQ174122 (1503; 31) strain PPT12-NE	MN227133 (1477) strain SRL1-PA	MN227135 (445)	MN227136 (795)	MN227134 (1450)	
'*Ca.* P. australasia'	Y10097 (1521; 11) strain PpYC	JQ868448 (1505; 48) strain TBB	JQ824250 (385)	EU168729 (482)	EF193373 (1294)	
'*Ca.* P. cirsii'	KR869146 (1498; 31) strain CirYS	KU557489 (462)				
'*Ca.* P. convolvuli'	JN833705 (1496; 31) strain 57/11	OK127877 (898)	OK127878 (1417)			
'*Ca.* P. cynodontis'	AJ550984 (1499; 29) strain BGWL-C1	KP019340 (1499; 31) strain 305/13	KP019342 (1528)			
'*Ca.* P. fragariae'	HM104662 (1502; 31) strain Straw	MK501641 (1531; 20) strain GBFC_SY_01	MN914137 (1122)			
'*Ca.* P. fraxini'	AF092209 (1462; 36) strain AshY=AshY1	GU004329 (1250)	KJ939978 (552)			
'*Ca.* P. japonicum'	AB010425 (1521; 10) strain JHP	AB738739 (1237)	AB746432 (1611)			
'*Ca.* P. hispanicum'	AF248960 (1527; 6) strain MPV	EU168753 (482)	GU004336 (1235)	EF193365 (1126)	KT444668 (552)	
'*Ca.* P. luffae'	AF248956 rRNAa	AF353090 rRNAb (1464; 69) strain LfWB	AF086617 (1188)	GU004319 (1257)		
'*Ca.* P. malaysianum'	EU371934 (1523; 6) strain MaPV	FJ755005 (482)				

'*Ca.* P. noviguineense'	LC228755 (1480; 55) strain BCSBoR	LC228769 (1247; 337–1584)	LC228762 (1439)			
'*Ca.* P. prunorum'	AJ542545 (1516; 7) strain ESFY-G2	JQ868450 (1494; 28) strain LNp	JQ824235 (385)			
'*Ca.* P. rhamni'	X76431 (1473; 47) strain BWB	KF498659 (1073)	JQ868449 (1494; 28) strain RhCa	JQ824207 (391)	KJ462067 (559)	
'*Ca.* P. rubi'	AY197648 (1529; 6) strain RuS	FN561887 (925)	KJ462043 (596)	AY197696 (1412)	FN562164 (797)	
'*Ca.* P. trifolii'	AY390261 (1531; 6) strain CP	KJ462045 (559)	GU004315 (1262)	AY197668 (1154)		

Note:
nt stands for nucleotide.
start denotes the starting nucleotide position of the sequence.
The data is based on the table from the reference "Bertaccini *et al.,* Int. J. Syst. Evol. Microbiol. 2022;72: 005353".

Table 4 : New officially proposed '*Candidatus* Phytoplasma' species GenBank numbers, available acronyms of the phytoplasma strains and nucleotide length, span and, for the 16S rRNA gene, the starting nucleotide are reported.

Phytoplasma	Full 16S rRNA (nt; start)	tufB (nt; span)	secA (nt; span)	secY (nt; span)	rplV-rpsC (nt; span)
'*Ca.* P. cocostanzaniae'	X80117 (1524 nt; 5) strain LD				
'*Ca.* P. palmae'	U18747 (1524 nt; 9)	VBRA02000009 (1544 nt; 3425–4968)	VBRA02000007 (1191 nt; 23653–24843)	VBRA02000009 (2418 nt; 171796–174213)	VBRA02000009 (1254 nt; 29596–30849)

Note:
nt stands for nucleotide.
start denotes the starting nucleotide position of the sequence.
The data is based on the table from the reference "Bertaccini *et al.,* Int. J. Syst. Evol. Microbiol. 2022;72: 005353".

Unique signature sequences when compared to the reference strain. The other 14 strains show sequence identities ranging from 98.05 to 98.50% when compared to the reference strain. Nucleotide sequences of the tufB, secA, secY, rplV-rpsC and groEL genes are available for the Texas Phoenix palm phytoplasma strain ACPD, with a draft genome available (GenBank accession number VBRA02000000). The only identified insect vector for '*Ca.* P. palmae' is Haplaxius (Myndus) crudus (Ogle *et al.,* 2005).

Comments on '*Candidatus* Phytoplasma' species listed in Table 2

'*Ca.* P. asteris' Strain MIAY, the reference strain described in 2004, is retained (Lee I-M. *et al.,* 2004). The whole genome sequences of three strains (OY-M, AY-WB and M3) and multiple draft assemblies are available. Strain OY-M was added as a complementary additional strain for other genes. Three hundred and sixty-six strains have 16SrRNA gene sequences covering >95% and sharing sequence identities ranging from 98.67% to 100% when compared to the reference strain MIAY (GenBank accession number M30790). Among strains having 100% sequence coverage, two share a 99.74% of identity, three 99.67%, two 99.61%, one 99.54% and one 99.48% when compared to the reference strain. Moreover, six strains show sequence identity ranging from 98.06 to 98.63%, while two strains show 97.77% sequence identity when compared to the reference strain. A large number of sequences are deposited for the 16SrRNA gene and several other conserved or housekeeping genes. '*Ca.* P. aurantifolia' This is the first '*Ca.* Phytoplasma' species formally described in 1995 (Zreik *et al.,* 1995); the reference strain WBDL is retained. Ninetyseven strains have 16SrRNA gene sequences covering >95% of the gene and share sequence identities ranging from 98.69% to 99.73% when compared to the reference strain WBDL (GenBank accession number U15442); 19 '*Ca.* P. australasia' strains share 98.32%–98.53% sequence identity with respect to the '*Ca.* P. aurantifolia' reference strain. Among the strains with a full sequence coverage, 25 have identities >99%when compared with the reference strain WBDL, but there are no strains with fully matching sequences. '*Ca.* P. australiense' Described in association with the Australian grapevine yellows (Davis *et al.,* 1997). The reference strain AUSGY has a partial 16SrRNA gene sequence (1375 nt; GenBank accession number L76865) and a partial tufB gene sequence available. The strain CaPaus whole genome sequence is available (GenBank accession number AM422018), and it is proposed as a complementary additional strain. Twelve strains have 16SrRNA gene sequences covering >95% of the gene and sharing sequence identities ranging from 98.68% to 99.93% compared to the CaPaus strain sequence. '*Ca.* P. mali' The apple proliferation agent, strain AP15, was described in 2004 (Seemüller *et al.,* 2004). The whole genome sequence of the severe strain AT is available. This strain encodes two identical 16SrRNA gene sequences (GenBank accession number CU469464). '*Ca.* P. mali' strains differ by up to 0.20% in their 16SrRNA sequences based on alignments with a coverage of at least 99%. Sequence identities >97.50% are available when compared with '*Ca.* P. pyri' and '*Ca.* P. prunorum'. '*Ca.* P. pyri' strain PD1 and '*Ca.* P. mali' strain AT show 98.60% identity on their 16SrRNA gene sequence and can be separated. A total of 20 '*Ca.* Phytoplasma' strains are deposited with sequences enclosing >95% coverage and showing identities ranging from 99.74% to 100% to the reference strain. However, 20 strains showing 98.22%–99.01% identity are classified within diverse '*Ca.* Phytoplasma' species ('*Ca.* P. pyri' and '*Ca.* P. prunorum'). On the aligned gene sequences, strain PD1 shows lower identities compared to the AT strain for the genes tufB (95.00%), secA (93.00%), secY (94.00%), rplV-rpsC (87.00%) and groEL (96.00%). '*Ca.* P. mali' strain differentiation is also possible based on their different insect vectors.

'*Ca.* P. meliae' It was described in Argentina in 2016 with four strains identified with the prefix ChTY (Fernández *et al.,* 2016). A comparison of the 16SrRNA gene sequences described in that manuscript showed a sequence identity of 99.82% to '*Ca.* P. hispanicum', the complete 16SrRNA gene sequence lowered the identity to 98.95%. Five strains are deposited with sequences covering >95%and showing identities ranging from 99.45% to 99.86% to the reference strain; however, six strains showing 98.82%–99.46% identity are classified within '*Ca.* P. hispanicum'. The secA and rplV-rpsC gene sequences show identities of 93.75% and 95.14% respectively, indicating a clear taxon separation compared with '*Ca.* P. hispanicum'. Having the draft genome sequence of strain ChTY-XIII-Mo (GenBank accession number NZ_JACAOD020000000) available, comparisons of its secY and groEL gene sequences to those of the MPV strain (GenBank accession numbers GU004336 and KT444668) indicate 90.08% and 93.84% sequence identity respectively, supporting it as the retained reference strain. '*Ca.* P. pini' It was described in 2005 with the Spanish strain Pin127S (GenBank accession number AJ632155) as the reference strain. Over 35 16SrRNA genes of various lengths (484–1250bp) are available. The three longer sequences share identities over 99.00% (99.74%–99.93%), except for the North American '*Ca.* P. pini' strains (GenBank accession numbers KU242428 and VIAE01000001) that share 98.50% sequence identity. '*Ca.* P. pini' shares the highest 16SrRNA sequence identity compared to '*Ca.* P. cynodontis' and '*Ca.* P. palmae' with values of about 93.00%–94.00%. secA and tufB gene sequences for some strains are deposited. A draft genome sequence of the North American '*Ca.* P. pini' strain MDPP is also available (Cai *et al.,* 2020). '*Ca.* P. phoenicium' The A4 reference strain is retained (Verdin *et al.,* 2003). Strain SA213 (draft genome available with a partial 16SrRNA gene sequence) was added as a complementary additional strain for the other genes. For strain SA213, 16SrRNA and tufB gene sequences were obtained from PCR products, while nucleotide sequences of the secA, secY, rplV-rpsC and groEL genes were retrieved from the draft genome. Fifty-one strains have 16S rRNA gene sequences covering >95% of the gene and sharing sequence identities from 98.71% to 99.93% compared to the reference strain (A4) sequence (GenBank accession number AF515636). Twenty-seven strains share sequence identities ranging from 97.54% to 98.61% compared with the reference strain. '*Ca.* P. pruni' Proposed in 2013 (Davis *et al.,* 2013), it is one of the phytoplasmas with the highest number of described strains (203), showing 98.80%–100% nucleotide identities compared to the reference strain. Four strains show 98.09%, 98.34%, 97.87% and 97.96% sequence identity compared to the reference strains. It is widely distributed, being mostly described in the American continent. '*Ca.* P. pyri' The reference strain PD1 (GenBank accession number AJ542543) was described along with '*Ca.* P. mali' and '*Ca.* P. prunorum' (Seemüller *et al.,* 2004) and shares 16SrRNA gene sequence identity >97.50% when compared with both. Thirty-four strains have >95% sequences available showing 99.14%–100% sequence identity compared to the reference strain. Among strains classified within '*Ca.* P. mali' and '*Ca.* P. prunorum', 64 show 98.68%–99.27% and seven

97.50%–98.64% sequence identity compared to the reference strain. The closely related '*Ca.* P. pyri' PD1 and '*Ca.* P. mali' AT strains can be separated by the sequences of additional genetic markers as mentioned for '*Ca.* P. mali'. Differentiation is also possible based on differential insect vector transmission. '*Ca.* P. sacchari' Described from sugarcane in India (Kirdat *et al.,* 2021), the reference strain SCGS is retained. Thirty strains have 16SrRNA gene sequences covering >95% of the gene and share sequence identities ranging from 98.69% to 99.93% compared to the reference strain SCGS (GenBank accession number MN889545); however, no strains show 100% sequence identity to it. Eleven strains having >95% of the sequence available show 98.79%–98.99% sequence identity compared to the reference strain. Seventeen strains are assigned to '*Ca.* P. cynodontis'. A draft genome assembly is available for the reference strain SCGSR (GenBank accession number VWXM00000000), which has a genome-wide ANI value of 79.42% compared to the closest relative with a genome assembly available, '*Ca.* P. cynodontis' strain LW01 (GenBank accession number VWOH00000000). For comparison of the groEL gene, the partial sequence available from SCGS shares 86.17% sequence identity with that of '*Ca.* P. cynodontis' strain 305/13. '*Ca.* P. solani' The reference strain STOL was described in 2013 and is retained (Quaglino *et al.,* 2013). For strain 284/09, all gene sequences are available and retrieved from its genome sequence (GenBank accession number FO393427). Seventy-two strains have 16S rRNA gene sequences covering >95% of the gene and share sequence identities ranging from 99.12% to 99.93%, compared to the reference strain sequence (GenBank accession number AF248959); one strain shows 98.17% sequence identity compared to the reference strain. Some strains [142/09, GenBank accession number JQ730739 (98.05%); 429/19, GenBank accession number MT157232 (98.04%); 204/10, GenBank accession number JQ730744 (98.04%); 198/10, GenBank accession number JQ730743 (98.04%); 224/09, GenBank accession number JQ730742 (98.04%); G66, GenBank accession number JN887313 (98.04%); 241/13, GenBank accession number KF907506 (98.04%); Conv2/2010-Bg, GenBank accession number JN561702 (98.03%); 161/16, GenBank accession number KY579338 (98.02%)] share a sequence identity >98%also with the '*Ca.* P. australiense' strain CaPaus (AM422018). The '*Ca.* P. solani' and '*Ca.* P. australiense' strains are, however, clearly distinct based on sequence identity comparisons of their tufB (82.00%–87.00%), rplV-rpsC (75.00%–82.00%) and secY (55.00%–75.00%) genes. '*Ca.* P. tritici' Recently described from wheat in China (Zhao *et al.,* 2021). The reference strain WBD has 98.68%–99.93% sequence identity compared with 434 '*Ca.* P. asteris' strains; therefore, it does not have the required threshold to be described as species based on the 16S rRNA gene. The proposal of this new taxon was based on its unique vectorship, a distinctive symptomatology in its predominant plant host, and 95% of the gene and sharing sequence identities of 99.53%–99.93% compared with strain EY1 (GenBank accession number AY197655). A total of 69 strains showing sequence identities ranging from 98.82% to 99.80% were assigned to other 'Ca. Phytoplasma' species. Strains of '*Ca.* P. ulmi' with rplV-rpsC gene sequences covering >89% share a sequence identity higher than 99.25% compared to strain EY1, which shares a rplV-rpsC gene sequence identity between 97.33% and 97.50%, and from 96.0% to 96.25% with strains of '*Ca.* P. rubi' and '*Ca.* P. ziziphi' respectively, with a sequence coverage of 100%. Strains of '*Ca.* P. ulmi' with secY gene sequences covering >77% share a sequence identity higher than 97.21% compared to the reference strain EY1; whereas '*Ca.* P. ulmi' strain EY1 shares a secY gene sequence identity of 92.37% and between 88.57% and 88.84% with strains of '*Ca.* P. rubi' and '*Ca.* P. ziziphi', respectively, with a sequence coverage of 100%. '*Ca.* P. ziziphi' Among the strains deposited with 100% sequence coverage, seven show 99.93% sequence identity compared to the reference strain JWB-G1 (GenBank accession number AB052876) (Jung *et al.,* 2003). Thirty-three strains with >95% sequence coverage show identity percentages ranging from 99.54% to 100% compared to the reference strain. Another 47 strains having a threshold ranging between 98.69% and 99.35% were included within '*Ca.* P. ulmi', '*Ca.* P. rubi' and 'flavescence dorée' but should be reclassified due to substantial differences in other gene sequences. COMMENTS ON '*CA.* PHYTOPLASMA' SPECIES LISTED IN TABLE 3 '*Ca.* P. americanum' Four strains including the reference strain are available, all from the USA, showing 16SrRNA gene sequence identities ranging from 99.67% to 99.87% (GenBank accession numbers DQ174118, DQ174120, MN227133 and DQ174121). '*Ca.* P. australasia' Originally described in New Zealand (White *et al.,* 1998), but it is also distributed in the Asian and North African continents. Presently 249 strains show sequence identities ranging from 98.65% to 100%. A total of 97, 94 and 74 strains classified as '*Ca.* P. aurantifolia' share sequence identities >97.5%, 98.00% and 98.65%, respectively, compared with '*Ca.* P. australasia' reference strain PpYC (GenBank accession number Y10097). GenBank shows 53 strains with a full-length 16SrRNA gene sequence identity ranging between 99.22% and 99.94%, compared to the reference strain. Six strains were erroneously attributed to '*Ca.* P. aurantifolia' and one to '*Ca.* P. australiense', while many other strains were attributed to the '*Ca.* P. australiense' due to a shorter sequence coverage resulting in identity values >98%. However, a distinction can be achieved by comparing additional genes (Tables 2 and S2). For the tufB gene, the majority of GenBank sequences fully match that of '*Ca.* P. australasia', but were misclassified as '*Ca.* P. aurantifolia', while the actual identity threshold for '*Ca.* P. aurantifolia' starts from 94.03%. Similarly, for secA and ribosomal protein (rp) genes, hundreds of strains in GenBank described as '*Ca.* P. aurantifolia' are misclassified showing sequence identities above 99.00%, compared to the corresponding genes of '*Ca.* P. australasia'.

'*Ca.* P. cirsii' It was described in the Czech Republic in association with yellowing, stunting and proliferation of creeping thistle and dahlia (Šafářová *et al.,* 2016). Two strains, including the reference strain CirYS (GenBank accession number KR869146), are available. Compared to the reference strain CirYS, strains CirYS1 and DahIP have 16SrRNA gene sequence identity percentages of 100.00% and 99.93%, respectively. The nucleotide sequence of the secA gene is available for the reference strain. '*Ca.* P. convolvuli' It was described as associated with bindweed yellows in several European countries (Martini *et al.,* 2012). The reference strain BY-S57/11 (GenBank accession number JN833705) is retained. Nine strains have 16SrRNA gene sequences covering >95% of the gene, sharing sequence identity ≥99.93%–100% compared to the reference strain. In GenBank, three phytoplasma strains associated with Carica papaya bunchy top in Nigeria showed a 98.73% sequence identity compared to the '*Ca.* P. convolvuli' reference strain. '*Ca.* P. cynodontis' It was described in association with the Bermuda grass white leaf (BGWL) disease and includes strains from Asian and European countries (Marcone *et al.,* 2004). The reference strain BGWL-C1 (GenBank accession number AJ550984) is retained, and strain 305/13 (GenBank accession number KP019340) is proposed as a complementary additional strain for the availability of its groEL gene. Thirty-five strains with 16SrRNA gene sequence coverages of >95% share sequence identities ranging from 98.66% to 100% compared to the reference strain. Among them, four strains from Italy, Albania and Iran show a 100% sequence identity, compared to each other and to the reference strain. One strain shows 98.37% and 15 strains show sequence identity percentages ranging from 98.33% to 98.84% compared to the reference strain. Other strains have 16SrRNA sequence identity ranging from 98.00% to 99.87% compared to the reference strain, including four strains assigned to '*Ca.* P. sacchari' and one strain assigned to '*Ca.* P. oryzae'. Comparing the groEL gene, '*Ca.* P. cynodontis' and '*Ca.* P. sacchari' can be distinguished (sequence identity 83.80%). '*Ca.* P. fragariae' It was described as associated with strawberry yellows in Lithuania (Valiunas *et al.,* 2006). Strain StrawY is the reference strain (GenBank accession number DQ086423) and it is retained. The strain GBFC_SY_01 was added as a complementary additional strain. A total of 10 strains had 16SrRNA sequence coverage >95%and share sequence identities from 99.46% to 99.93% compared to the reference strain. Four strains show 98.23%–98.63% and one shows 97.67% sequence identity to the reference strain. The species '*Ca.* P. fragariae' is closely related to '*Ca.* P. japonicum' and it was also detected in potato plants in China. '*Ca.* P. fraxini' The reference strain AshY1 is retained, with secY and groEL gene sequences available (Griffiths *et al.,* 1999). There are nine strains with 16SrRNA sequences covered for over >95%and sequence identities from 99.53% to 99.93% compared to the reference strain. There are eight strains showing identities ranging from 98.01% to 98.61% to the reference strain and five strains with identities ranging between 97.61% and 97.81%. Two complete sequences and four partial sequences of rplV-rpsC genes are available showing nucleotide identities over 99.45% among them. '*Ca.* P. fraxini' strains were mainly identified in the American continent. Further studies are necessary to clarify the identity of a phytoplasma detected in Crotalaria juncea in Brazil (Wulff *et al.,* 2015) showing 97.6% 16SrRNA sequence identity (GenBank accession number KF941132) and 92.03% for the rplV-rpsC genes (GenBank accession number KJ806620) to the reference strain. '*Ca.* P. japonicum' Identified in Japan (Sawayanagi *et al.,* 1999) (reference strain JHP, GenBank accession number AB010425); one strain was detected in China in Sophora japonica (GenBank accession number FJ685751). Nucleotide sequences of the genes secY and groEL are available for the reference strain. '*Ca.* P. hispanicum' Identified in periwinkle in Mexico (Davis *et al.,* 2016) it has >98% 16SrRNA gene identity to '*Ca.* P. meliae'. The reference strain MPV, for which the sequences of the genes rplV-rpsC, secY, secA, and groEL are available, is retained. A total of six strains had 16SrRNA gene sequences covering >95% of the gene sharing sequence identities of 98.74%–99.47%. Moreover 98.53% sequence identity is present for one '*Ca.* P. hispanicum' strain, while 98.82%–98.89% sequence identity corresponds to four strains of '*Ca.* P. meliae'. '*Ca.* P. luffae' It was identified in Taiwan

(Davis *et al.,* 2017), there are now 13 16SrRNA gene sequences longer than 1200bp deposited in GenBank showing 99.87%–99.93% sequence identity compared with the reference strain LfWBR. However, LfWBR was lost, and another strain collected in Taiwan, NCHU2019, with a complete genome sequence available (GenBank accession number CP054393) is proposed as an additional complementary strain. NCHU2019 and LfWBR share 100% sequence identity of their 16SrRNA and secY gene sequences, and a 97.58% sequence identity of their tufB gene sequences. '*Ca.* P. malaysianum' Described from Malaysia (Nejat *et al.,* 2013), one more strain is described from South Korea having 99.54% sequence identity to its 16SrRNA sequence. One partial sequence (482bp) of the secA gene is also available. '*Ca.* P. noviguineense' Described in Papua New Guinea in association with Bogia coconut syndrome (BCS) and banana wilt (BW) in coconut and banana plants, respectively (Miyazaki *et al.,* 2018). The reference strain is BCS-BoR from coconut (GenBank accession number LC228755). A total of 26 strains with 16SrRNA sequence coverage >95% share sequence identities from 99.66% to 100% compared to the reference strain. Sequence identities of both BCS and BW strains to other phytoplasma taxa are about 96% with maximum values of 96.08%, 95.91%, 95.20% for '*Ca.* P. palmae', '*Ca.* P. cocostanzaniae' and '*Ca.* P. palmicola', respectively. Another phytoplasma from the same island associated with arecanut yellow leaf disease showed high 16SrRNA gene sequence identity and is closely related to BCS-BoR. Additional sequence comparison of the secY (GenBank accession number LC228769) and rplV-rpsC genes (GenBank accession numbers LC228762) for strain BCS-BoR for strain BCS-G (GenBank accession number LC228763) and for strain BCS-S (GenBank accession number LC228764) showed 100% sequence identity compared to the reference strain BCS-BoR. '*Ca.* P. prunorum' The reference strain EFSY-G1 (GenBank accession number AJ542544) was described with '*Ca.* P. mali' and '*Ca.* P. pyri'. There are 32 strains with 16SrRNA gene sequences covering >95% with identity percentages from 99.50% to 100% compared to the reference strain. Moreover, 63 '*Ca.* P. pyri' and '*Ca.* P. mali' strains show sequence identities from 98.67% to 99.14%, and 13 show identities between 98.31% and 98.62% to the reference strain. However, differentiation of these '*Ca.* Phytoplasma' species is possible based on insect vector differential transmission. '*Ca.* P. rhamni' It was identified in 1994 in buckthorn plants in south-west Germany. Nine additional '*Ca.* P. rhamni' 16SrRNA sequences from Austria (GenBank accession number KF498655), Germany (GenBank accession numbers KF498651-52, JQ868449), Serbia (GenBank accession numbers KF498656-58) and Switzerland (GenBank accession numbers KF498653-54) are available which are fully identical to each other but differ from the reference strain BAWB. The complete rplV and a partial sequence of the rpsC gene are available for eight strains (GenBank accession numbers KF498659–66) all showing a high sequence identity. SecA and tufB gene sequences are also available. 'Ca. P. rubi' Described based on biological properties and seven additional strains have been identified with 16SrRNA sequence identity between 99.83% and 99.88% compared to the reference strain (Malembic-Maher *et al.,* 2011). There are 86 strains with sequence identity ranging from 98.66% to 99.52%, which are classified within different '*Ca.* Phytoplasma' species, or taxa not yet named as '*Ca.* Phytoplasma' species. A threshold for its differentiation and support can be settled for secA (98.37%) and secY (97.03%) genes. '*Ca.* P. trifolii' Associated with clover proliferation in Trifolium hybridum plants (Hiruki *et al.,* 2004). The reference strain CP (GenBank accession number KJ462045) is retained. For this strain the sequences of secY, secA and rplV-rpsC genes are available. Seventy-six strains have 16SrRNA gene sequences covering >95% of the gene sharing sequence identities between 98.77% and 100%, three strains range from 98.15% to 98.63% and one has 97.98% sequence identity compared to the reference strain. There are 13 secA gene sequences available showing sequence identities between 99.10% and 99.28% to the reference strain CP. Comments on '*Ca.* Phytoplasma' species described only based on the 16SrRNA gene '*Ca. P. allocasuarinae*' Only the reference strain sequence is available from Australia. A strain from a Empoasca species in Cuba (GenBank accession number AY725236) shows 98.52% sequence identity to it (with a 99% coverage of the 16SrRNA sequence). '*Ca.* P. balanitae' The reference strain BltWB (GenBank accession number AB689678) was described in Myanmar, infecting the unique species *Balanites triflora*, endemic in that state. A total of 13 strains with >95% 16SrRNA gene sequence coverage is available and share 99.41%–99.80% sequence identity to the reference strain. It was attributed to several other phytoplasma strains detected in other plant species in India. This attribution is not correct since, following the previous rules, it must only be used for this phytoplasma when it is infecting *B. triflora*, plant species that is not present in India.

'*Ca. P. brasiliense*' The reference strain HibWB26 (GenBank accession number AF147708) has 16SrRNA gene sequence identities ranging from

88.80% to 96.40% when compared with other '*Ca.* Phytoplasma' species (Montano *et al.,* 2001). A total of 10 strains with >95% coverage of their 16SrRNA gene sequences is available and share 98.73%–99.93% sequence identity to the reference strain. Further 16SrRNA gene sequences are available for the Peruvian papaya phytoplasma (GenBank accession number KX810334-36) and grapevine (GenBank accession numbers KX670807-9) phytoplasma strains that show 98.64% sequence identity with the reference strain. A groEL gene sequence of 555 nt of the strain identified in papaya in Peru is available (GenBank accession number MH279494). Phytoplasmas were reported with 16SrRNA gene sequence identities of 98.84% from Guazuma ulmifolia (GenBank accession number HQ258882) in Costa Rica, 99.68% from Sida rhombifolia (GenBank accession number HQ230579) and 99.94% from Crotalaria juncea (GenBank accession number KF878382) in Brazil. A '*Ca.* P. brasiliense' strain found in hibiscus in Egypt (GenBank accession number KF716175) showed 99.60% sequence identity with the reference strain. Further strains were identified in peach in Azerbaijan (GenBank accession number FR717540) and in *Catharanthus roseus* in Costa Rica (GenBank accession number MH428963). '*Ca.* P. caricae' it was identified from papaya plants in Cuba in 2005 and the reference strain is PAY (GenBank accession number AY725234). It shares 95.80% sequence identity of the 16SrRNA gene with '*Ca.* P. graminis'. '*Ca.* P. castaneae' It was described from infected chestnut in South Korea (Jung *et al.,* 2002) and two more strains (GenBank accession numbers MW264918 and EU599362) were reported from China. '*Ca.* P. costaricanum' It was described as associated with phytoplasma diseases in soybean, sweet pepper and passionfruit in Costa Rica. The retained reference strain is SoyST1c1 (GenBank accession number HQ225630). Seventeen strains have 16SrRNA gene sequence coverage >95%and share sequence identities between 99.15% and 99.61% compared to the reference strain. '*Ca.* P. dypsidis' It was recently described in Australia from dying ornamental palms belonging to several species. Six strains with coverage >95%show sequence identities between 99.83% and 99.88% compared to the reference strain RID7692 (GenBank accession number MT536195). The closest phytoplasma is '*Ca.* P. cocostanzaniae' with a sequence identity 95%show identities between 99.61% and 99.74% and one strain has identity of 98.43% compared to the reference strain. '*Ca.* P. lycopersici' It was described in Bolivia (Arocha *et al.,* 2007) in tomato and Morrenia variegata. The reference strain is Santa Cruz (GenBank accession number EF199549). The 16SrRNA gene sequence of one strain is deposited in GenBank sharing 97.51% sequence identity with some '*Ca.* P. asteris' strains. '*Ca.* P. omanense' It was identified in Oman in Cassia italic, the reference strain IM-4 (GenBank accession number EF666054) has 99.64% sequence identity compared to the other three strains (GenBank accession numbers EF666051-53); 99.58% sequence identity is present in the only strain with >95% of sequence available. A strain from Australia from a Vigna species (GenBank accession number AJ289195) shares 98.04% sequence identity to the reference strain. '*Ca.* P. oryzae' It was reported from infected rice collected in Japan; the reference strain is RYD-J R. Strain RYD-Th, from Thailand (GenBank accession number AB052873), shares 99.20% 16S rRNA gene identity with RYD-J R. Two other strains reported as '*Ca.* P. oryzae' have a draft genome sequence available, both were collected from infected Napier grass, including Mbita1 (GenBank accession number LTBM00000000) from Kenya and NGS-S10 (GenBank accession number JHUK00000000) from Ethiopia. However, Mbita1 lacks a 16SrRNA gene in the draft genome sequence while the 16SrRNA gene of NGS-S10 shares 97.38% sequence identity with RYD-J R. Three more strains are available with a 16SrRNA gene sequence identity of 99.93%, 99.78% and 99.56%, respectively, to that of the NGS-S10 strain. More strains have a sequence identity of '*Ca.* P. palmicola' It was described from coconut palms with symptoms of lethal yellowing in Mozambique, the reference strain is LYDM-178 (GenBank accession number KF751387). The 16SrRNA gene sequence alignments confirmed the identity with those of Awka wilt disease in Nigeria (GenBank accession number Y14175), and 99.00%–99.60% sequence identity with those of the Cape St Paul Wilt disease in Ghana (GenBank accession numbers Y13912 and JQ868442) and Côte d'Ivoire, CILY, (GenBank accession numbers KC999037, KF364359, KF387570 and KF419286). A total of 24 sequences with identities ranging from 99.28% to 100% compared to the reference strain is available. Strains from Côte d'Ivoire and Ghana can be differentiated by single nucleotide polymorphisms in their 16SrRNA gene sequences. There are 32 secA sequences of 627bp (GenBank accession numbers LR029104–LR029135) corresponding to Mozambican strains, but none for the LYDM reference strain. Other secA sequences of strains from Nigeria include GenBank accession numbers LR029136–LR029139. Ghana/Côte d'Ivoire strain sequences for the genes tufB (GenBank accession number JQ824292, 391bp) and rpIV-rpsC (GenBank accession numbers KU925788–KU925794, 825 nt; GenBank accession numbers LR028744–LR028839, 321 nt) are available. ‘*Ca.* P. spartii’ It was only identified in Spartium junceum in Europe. The reference strain is SpaWB (GenBank accession number X92869). There are six strains deposited with sequence identities ranging from 98.97% to 99.84% to the reference strain. ‘*Ca.* P. stylosanthis’ It was described in Australia in diverse plant species. Among the four sequences available, only one with >95% 16S rRNA gene sequence coverage shares 99.94% sequence identity with the reference strain. The following GenBank accession numbers correspond to the reference strain: MT431550, 16SrRNA gene; MT432821, partial secA gene; MT432813, partial tufB gene; MT461153, partial rps19-rpl22-rps3 gene. ‘*Ca.* P. tamaricis’ It was identified in China, one more strain (GenBank accession number MW447513) is available sharing a 99.67% sequence identity to it, but with only 67% coverage. ‘*Ca.* P. sudamericanum' It was identified in passion fruit plants in Brazil; the reference strain is PassWB-Br3 (GenBank accession number GU292081) and has 97.5% 16SrRNA gene sequence identity with existing species, and it was clearly proven that it represented an ecologically separated population. For such cases, the description of two different ‘*Ca.* Phytoplasma’ species was recommended only when all three of the following conditions apply (i) the two phytoplasmas are transmitted by different vectors; (ii) the two phytoplasmas have a different natural plant host (or, at least, their behaviour is significantly different in the same plant host); (iii) there is evidence of significant molecular diversity, achieved by either hybridization to cloned DNA probes, serological reaction or a PCR-based assay. To date, more than 30 phytoplasma genomes (completed and drafted) have been published. Two species have been named based on their ANI value of 98.65%, the following threshold values based on housekeeping genes should be used to support their effective distinction: 97.6% for groEL gene, 97.5% for tuf and rp genes, 95.7% for secA gene and 95.0% for secY gene. The new thresholds include a 16SrRNA gene sequence identity of 98.65%, a genome ANI of 95%, and two among five suggested housekeeping genes. For example, if the 16SrRNA gene sequence identity for a given phytoplasma is 98.65% and the genome ANI is 98.65% and the whole genome sequence is unavailable. There are no guidelines for selecting or validating the name of a particular ‘*Ca.* Phytoplasma’ species. This should follow the required grammatical rules, the specific geographic distribution, the major or first plant host where the phytoplasma was identified. The revised guidelines support all the previously assigned ‘*Ca.* Phytoplasma’ species, except four that must be adjusted to fit the revised guidelines. Previous International Research Programme on Comparative Mycoplasmology guidelines should be followed if relevant for a complete description of the new ‘*Ca.* Phytoplasma’ species when not conflicting with the present revised guidelines.

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