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'*Candidatus* Phytoplasma', a taxon for the wall-less, non-helical prokaryotes that colonize plant phloem and insects

The IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma taxonomy group

Correspondence
G. Firrao
firrao@uniud.it

The trivial name 'phytoplasma' has been adopted to collectively name wall-less, non-helical prokaryotes that colonize plant phloem and insects, which were formerly known as mycoplasma-like organisms. Although phytoplasmas have not yet been cultivated *in vitro*, phylogenetic analyses based on various conserved genes have shown that they represent a distinct, monophyletic clade within the class *Mollicutes*. It is proposed here to accommodate phytoplasmas within the novel genus '*Candidatus* (*Ca.*) Phytoplasma'. Given the diversity within '*Ca.* Phytoplasma', several subtaxa are needed to accommodate organisms that share $<97{\cdot}5\,\%$ similarity among their 16S rRNA gene sequences. This report describes the properties of '*Ca.* Phytoplasma', a taxon that includes the species '*Ca.* Phytoplasma aurantifolia' (the prokaryote associated with witches'-broom disease of small-fruited acid lime), '*Ca.* Phytoplasma australiense' (associated with Australian grapevine yellows), '*Ca.* Phytoplasma fraxini' (associated with ash yellows), '*Ca.* Phytoplasma japonicum' (associated with Japanese hydrangea phyllody), '*Ca.* Phytoplasma brasiliense' (associated with hibiscus witches'-broom in Brazil), '*Ca.* Phytoplasma castaneae' (associated with chestnut witches'-broom in Korea), '*Ca.* Phytoplasma asteris' (associated with aster yellows), '*Ca.* Phytoplasma mali' (associated with apple proliferation), '*Ca.* Phytoplasma phoenicium' (associated with almond lethal disease), '*Ca.* Phytoplasma trifolii' (associated with clover proliferation), '*Ca.* Phytoplasma cynodontis' (associated with Bermuda grass white leaf), '*Ca.* Phytoplasma ziziphi' (associated with jujube witches'-broom), '*Ca.* Phytoplasma oryzae' (associated with rice yellow dwarf) and six species-level taxa for which the *Candidatus* species designation has not yet been formally proposed (for the phytoplasmas associated with X-disease of peach, grapevine flavescence dorée, Central American coconut lethal yellows, Tanzanian lethal decline of coconut, Nigerian lethal decline of coconut and loofah witches'-broom, respectively). Additional species are needed to accommodate organisms that, despite their 16S rRNA gene sequence being $>97{\cdot}5\,\%$ similar to those of other '*Ca.* Phytoplasma' species, are characterized by distinctive biological, phytopathological and genetic properties. These include '*Ca.* Phytoplasma pyri' (associated with pear decline), '*Ca.* Phytoplasma prunorum' (associated with European stone fruit yellows), '*Ca.* Phytoplasma spartii' (associated with spartium witches'-broom), '*Ca.* Phytoplasma rhamni' (associated with buckthorn witches'-broom), '*Ca.* Phytoplasma allocasuarinae' (associated with allocasuarina yellows), '*Ca.* Phytoplasma ulmi' (associated with elm yellows) and an additional taxon for the stolbur phytoplasma. Conversely, some organisms, despite their 16S rRNA gene sequence being $<97{\cdot}5\,\%$ similar to that of any other '*Ca.* Phytoplasma' species, are not presently described as *Candidatus* species, due to their poor overall characterization.

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Abbreviations: *Ca.*, *Candidatus*; IRPCM, International Research Programme for Comparative Mycoplasmology.

This paper is dedicated to the memory of Monique Garnier-Semancik, fine scientist and friend.

INTRODUCTION

Evidence that some plant diseases were associated with phloem colonization by prokaryotes that morphologically resembled mycoplasmas was first presented by Doi *et al.* (1967). Since then, several hundred plant syndromes have been associated with and thought to be caused by so-called

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'mycoplasma-like organisms' [reviewed by McCoy *et al.* (1989) and Seemüller *et al.* (1998)]. Due to their inability to grow *in vitro*, they were poorly characterized until the advent of molecular biology. Lately, rRNA gene sequencing has provided evidence that the non-spiroplasma, wall-less prokaryotes that colonize plant phloem and insects constitute a large, monophyletic group within the class *Mollicutes* (Lim & Sears, 1989; Kuske & Kirkpatrick, 1992; Sears & Kirkpatrick, 1994). Therefore, at the 9th Congress of the International Organization of Mycoplasmology in 1992, the Phytoplasma Working Team of the International Research Project for Comparative Mycoplasmology (IRPCM) adopted the trivial name 'phytoplasma' to identify prokaryotes that belong to this group and to represent its present composition (International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of *Mollicutes*, 1993). Since then, several distinct taxa have been described as '*Candidatus* (*Ca.*) Phytoplasma aurantifolia' (Zreik *et al.*, 1995), '*Ca.* Phytoplasma australiense' (Davis *et al.*, 1997), '*Ca.* Phytoplasma australasia' (White *et al.*, 1998), '*Ca.* Phytoplasma fraxini' (Griffiths *et al.*, 1999), '*Ca.* Phytoplasma japonicum' (Sawayanagi *et al.*, 1999), '*Ca.* Phytoplasma brasiliense' (Montano *et al.*, 2001), '*Ca.* Phytoplasma castaneae' (Jung *et al.*, 2002), '*Ca.* Phytoplasma phoenicium' (Verdin *et al.*, 2003), '*Ca.* Phytoplasma ziziphi' (Jung *et al.*, 2003a), '*Ca.* Phytoplasma oryzae' (Jung *et al.*, 2003b) and '*Ca.* Phytoplasma ulmi' (Lee *et al.*, 2004b), but a general description of the comprehensive taxon '*Ca.* Phytoplasma' itself has not yet been provided.

According to a recently adopted taxonomic rule (Murray & Schleifer, 1994; Murray & Stackebrandt, 1995), the properties of uncultured organisms should be recorded by a *Candidatus* designation. The scope of the present paper is to provide a formal description of the genus '*Ca.* Phytoplasma' and to summarize its species composition.

METHODS

16S rRNA gene sequence analysis. All GenBank records containing sequences that were defined, or could be referred to, as phytoplasma 16S rRNA genes were downloaded. Partial sequences that contained <1200 bp within the first 1400 positions at the 5′ end of the 16S rRNA gene were discarded. Seventy of the 194 selected sequences were aligned manually by using the sequence editor SEQPUP (Gilbert, 1996). Using the profile alignment option of the program CLUSTAL_X (Thompson *et al.*, 1997), the remaining 124 sequences were added to the manual alignment. Distances between sequences were calculated by using the method of Jukes & Cantor (1969) by the DNADIST program of the PHYLIP package (Felsenstein, 1995). The alignment was deposited in TreeBase (http://www.treebase.org/treebase/) under the accession no. S1048-1787. A dendrogram was constructed with the neighbour-joining method of Saitou & Nei (1987) with the CLUSTAL_X program (Thompson *et al.*, 1997) and was used to assist group definition. Sequence signatures were determined with ALISCAN (De Marta & Firrao, 2003). A second alignment that contains only the sequences of reference strains for the '*Ca.* Phytoplasma' species recognized in this work is available from TreeBase under the accession no. S1048-1788.

RESULTS AND DISCUSSION

Evidence for an unambiguously identifiable taxon to encompass all plant-pathogenic, non-helical *Mollicutes* has been provided by several independent phylogenetic analyses (Lim & Sears, 1989; Kuske & Kirkpatrick, 1992; Namba *et al.*, 1993b; Gundersen *et al.*, 1994; Kirkpatrick *et al.*, 1994; Sears & Kirkpatrick, 1994; Seemüller *et al.*, 1994, 1998, 2002; Smart *et al.*, 1994; Toth *et al.*, 1994; Tymon *et al.*, 1998; Lee *et al.*, 2000). Accordingly, phytoplasmas represent a clearly distinct, monophyletic cluster within the class *Mollicutes*.

Description of '*Candidatus* Phytoplasma'

'*Candidatus* Phytoplasma' (from *phytos*, Greek for plant and *plasma*, Greek for thing moulded) [(*Mollicutes*) NC; NA; O; NAS (GenBank accession no. M30790); oligonucleotide sequence of unique region of the 16S rRNA gene is CAAGAYBATKATGTKTAGCYGGDCT; P (Plant, phloem; Insect, salivary gland); M].

Morphology. '*Ca.* Phytoplasma' cells are surrounded by a single-unit membrane, lack rigid cell walls and are pleomorphic in shape (Doi *et al.*, 1967). When observed by transmission electron microscopy, they appear as rounded to filamentous, pleomorphic bodies with a mean diameter of 200–800 nm [reviewed by Kirkpatrick (1992) and Lee *et al.* (2000)].

Habitat. Organisms that belong to the genus '*Ca.* Phytoplasma' inhabit the phloem sieve elements (and, more rarely, also parenchymal cells) of vascular plants and the gut, haemolymph, salivary gland and other organs of sap-sucking insects. In insect hosts, they may cause premature mortality. In plant hosts, they may cause complex syndromes with specific symptoms, such as virescence, phyllody, sterility of flowers, proliferation of auxiliary or axillary shoots, abnormal elongation of internodes and many other, often less specific symptoms [reviewed by McCoy *et al.* (1989), Kirkpatrick (1992) and Lee *et al.* (2000)].

Antibiotic sensitivity. Members of the genus '*Ca.* Phytoplasma' are sensitive to antibiotics of the tetracycline group, but not to penicillin (Ishiie *et al.*, 1967).

Base composition of DNA. According to results from buoyant density centrifugation, the G+C content of '*Ca.* Phytoplasma' DNA is 23–29 mol% (Kollar & Seemüller, 1989; Sears *et al.*, 1989).

Chromosome size. The size of '*Ca.* Phytoplasma' chromosomes ranges from 530 kbp to 1350 bp, as estimated from mobility on PFGE (Neimark & Kirkpatrick, 1993; Marcone *et al.*, 1999).

Codon usage. Members of the genus '*Ca.* Phytoplasma' use UGA as a stop codon and not as a tryptophan codon,

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as in several other mycoplasmas (Lim & Sears, 1991; Toth *et al.*, 1994).

Sterols in cellular membrane. '*Ca.* Phytoplasma' membranes are resistant to digitonin and sensitive to hypotonic salt solutions, and are therefore similar to those of non-sterol-requiring mollicutes (Lim *et al.*, 1992).

rRNA. Specific nucleotide signatures that are characteristic of '*Ca.* Phytoplasma' are: A at position 242, T at position 286 and T at position 1247 (in the sequence with GenBank accession no. M30790). Oligonucleotides that have been shown to specifically target '*Ca.* Phytoplasma' 16S rRNA genes in PCRs have been published (Deng & Hiruki, 1991; Ahrens & Seemüller, 1992; Firrao *et al.*, 1993; Lee *et al.*, 1993b; Namba *et al.*, 1993a; Schneider *et al.*, 1993; Padovan *et al.*, 1995; Gundersen & Lee, 1996). All strains of '*Ca.* Phytoplasma' that have been investigated so far have two rRNA operons (Schneider & Seemüller, 1994; Firrao *et al.*, 1996b; Lauer & Seemüller, 2000; Padovan *et al.*, 2000; Marcone & Seemüller, 2001) and, usually, a single $tRNA^{Ile}$ in the spacer region between the 16S and 23S rRNA genes (Kuske & Kirkpatrick, 1992; Smart *et al.*, 1996). Heterogeneity of the two operons is apparent in some phytoplasmas (Lee *et al.*, 1993b; Firrao *et al.*, 1996a; Liefting *et al.*, 1996; Davis & Sinclair, 1998; Jomantiene *et al.*, 2002).

Species belonging to '*Ca.* Phytoplasma'

A similarity matrix that included all 16S rRNA gene sequences of named phytoplasma strains that were deposited in public databases was constructed. Sequences were ordered in a dendrogram and groups of sequences that shared 97·5 % or more similarity among themselves and <97·5 % similarity with any other sequence were identified. One sequence from each group was selected as representative and the corresponding strain is described as a subtaxon within the '*Ca.* Phytoplasma' taxon. According to Stackebrandt & Goebel (1994), 'at sequence homology values below about 97·5 % [in the 16S rRNA gene], it is unlikely that two organisms have more than 60 to 70 % DNA similarity and hence that they are related at the species level'.

A congruent amount of results has already been produced in recent years, showing that phytoplasma strains that have <97·5 % similarity in their 16S rRNA gene sequence actually represent different organisms, on the basis of phylogenetic analysis based on 16S rRNA gene sequences (Namba *et al.*, 1993b; Gundersen *et al.*, 1994; Seemüller *et al.*, 1994, 1998; Tymon *et al.*, 1998; Lee *et al.*, 2000), 16S–23S rRNA gene spacer sequences (Kirkpatrick *et al.*, 1994), 23S rRNA gene sequences (Guo *et al.*, 1998), ribosomal protein gene sequences (Jomantiene *et al.*, 1998; Lee *et al.*, 1998), transcription factor Tu gene (Schneider *et al.*, 1997a), restriction analysis of the same genes (Schneider *et al.*, 1993, 1997a, b; Vibio *et al.*, 1996; Lee *et al.*, 1998), RFLP of total DNA with chromosomal probes (Lee & Davis, 1988; Bertaccini *et al.*, 1990; Harrison *et al.*, 1991, 1992; Hibben *et al.*, 1991; Kuske *et al.*, 1991; Lee *et al.*, 1991, 1992a, b, 1993a; Daire *et al.*, 1992; Davis *et al.*, 1992a, b; Ahrens *et al.*, 1993; Mäurer *et al.*, 1993; Chen *et al.*, 1994; Griffiths *et al.*, 1994; Davis & Sinclair, 1998) and biological properties, such as insect vector specificity (Tsai, 1979; Shiomi & Sugiura, 1984; Kirkpatrick, 1992).

However, there is evidence that some groups that are defined at 97·5 % or higher 16S rRNA gene sequence similarity include phytoplasma strains with very different biological (i.e. insect vector), phytopathological (i.e. host plant specificity and symptomatology) and molecular (i.e. chromosome size) characteristics. These strains need to be distinguished taxonomically. It has been highlighted in the past that in several taxonomic groups, organisms with highly similar or even identical 16S rRNA gene sequences may nevertheless share <70 % DNA similarity (as estimated by DNA–DNA reassociation studies) and therefore belong to different species (Fox *et al.*, 1992; Stackebrandt & Goebel, 1994; Stackebrandt *et al.*, 2002; Botti & Bertaccini, 2003). In the case of the phytoplasmas, there are also practical reasons of concern, as organisms that share high 16S rRNA gene sequence similarity may cause different plant diseases, subject to quarantine regulations. Therefore, in addition to the minimal set of species belonging to '*Ca.* Phytoplasma' that are defined by 97·5 % 16S rRNA gene sequence similarity, more *Candidatus* species have been defined to distinguish between those organisms that proved to be significantly different on the basis of biological and genetic properties.

In order to prevent nomenclatural confusion that may arise from the description of poorly differentiated novel taxa, the Phytoplasma/Spiroplasma Working Team of the IRPCM (2000) suggested rules for the description of organisms as novel taxa within '*Ca.* Phytoplasma', as follows.

(a) The '*Ca.* Phytoplasma' species description should refer to a single, unique 16S rRNA gene sequence (>1200 bp). The strain from which this sequence was obtained should be named the 'reference strain' and not the 'type strain'. Strains in which even minimal differences in the 16S rRNA gene sequence from the reference strain are detected do not 'belong' to the *Candidatus* species, but are 'related' to it.

(b) In general, a strain can be described as a novel '*Ca.* Phytoplasma' species if its 16S rRNA gene sequence has <97·5 % similarity to that of any previously described '*Ca.* Phytoplasma' species.

(c) There are, however, cases of phytoplasmas that share >97·5 % of their 16S rRNA gene sequence, but clearly represent ecologically separated populations and, therefore, may deserve description as separate species. For such cases, description of two different species is recommended only when all three of the following conditions apply:

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Table 1. List of 16S rRNA gene sequences of strains related to '*Ca.* Phytoplasma' species

Refer to database records (accession numbers are given) for full references. The 16S rRNA group according to Lee *et al.* (2000) is given in parentheses in the first column.

Phylogenetic group	'*Candidatus* Phytoplasma' species	Reference of the species description paper	GenBank accession no.	Database entry description
Aster yellows group (16SrI)	'*Ca.* Phytoplasma asteris'	Lee *et al.* (2004a)	M30790	Mycoplasma-like organism (strain OAY)
			M86340	Mycoplasma-like sp.
			AF177384	Alfalfa stunt phytoplasma
			U96616	*Phytoplasma* sp. STRAWB2
			L33760	Tomato big bud mycoplasma-like organism
			AF268403	Aster yellows phytoplasma A isolate 98UW159
			AF268404	Aster yellows phytoplasma A isolate 98UW166A
			AF268405	Aster yellows phytoplasma O isolate 98UW166B
			AF503568	Aster yellows phytoplasma B
			AY075038	Mulberry dwarf phytoplasma
			AF217247	Potato purple top phytoplasma
			AF200431	*Cirsium* yellows phytoplasma
			AF245439	Aster yellows phytoplasma
			AF222064	Tomato big bud phytoplasma
			AF222063	Aster yellows phytoplasma
			AF222065	Clover phyllody phytoplasma strain CPh rrnA
			AF222066	Clover phyllody phytoplasma strain CPh rrnB
			X68373	Mycoplasma-like organism (substrain AAY)
			U89378	*Phytoplasma* sp.
			L33762	Clover phyllody mycoplasma-like organism
			D12569	Group I phytoplasma
			X68338	Mycoplasma-like organism (substrain ACLR)
			AF268408	Aster yellows phytoplasma A isolate 99UW111
			AF268409	Aster yellows phytoplasma B isolate 99UW108
			AF322644	Aster yellows phytoplasma strain AY1 clone 14 A
			AF291682	Carrot proliferation phytoplasma
			AF335107	*Rehmannia glutinosa* var. *purpurea* phytoplasma
			AF453328	*Phytoplasma* sp. PY1
			AF279271	Paulownia witches'-broom phytoplasma
			AF356846	Cactus phytoplasma 'Martinez-Soriano 2001'
			AF322645	Aster yellows phytoplasma strain AY1 clone 10
			L33767	Aster yellows mycoplasma-like organism
			AF268407	Aster yellows phytoplasma B isolate 99UW89
			AF268406	Aster yellows phytoplasma A isolate 99UW93
	'*Ca.* Phytoplasma japonicum'	Sawayanagi *et al.* (1999)	AB010425	*Phytoplasma* sp.

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Table 1. cont.

Phylogenetic group	'*Candidatus* Phytoplasma' species	Reference of the species description paper	GenBank accession no.	Database entry description
Peanut witches'-broom group (16SrII)	'*Ca.* Phytoplasma aurantifolia'	Zreik *et al.* (1995)	U15442	'*Candidatus* Phytoplasma aurantifolia'
			AB026155	*Phytoplasma* sp. Gph
			AF028813	Chinese pigeon pea witches'-broom phytoplasma
			AF200718	Cactus phytoplasma 'Martinez-Soriano 1999'
			X83432	*Mollicutes* sp. from *V. faba*
			X76433	*Mollicutes* (from *C. juncea*)
			Y16393	*Picris echiodes* phyllody phytoplasma
			AJ289191	Pigeon pea little leaf phytoplasma
			Y10096	*Mollicutes* sp. (associated with papaya mosaic disease)
			Y10097	*Mollicutes* sp. (associated with papaya yellows crinkle disease)
			L33765	Peanut witches'-broom mycoplasma-like organism
			Y08173	*Phytoplasma* sp. (tomato big bud, Australia)
			L33770	Sweet potato witches'-broom mycoplasma-like organism
			AJ289193	Sweet potato little leaf phytoplasma strain V4
			Y16390	Italian alfalfa witches'-broom phytoplasma
			AJ295330	Cocky apple witches'-broom phytoplasma
			AF320575	*Phytoplasma* sp. pathovar mosaic-inducing
			AF331973	Cactus witches'-broom phytoplasma
			AF438413	Alfalfa witches'-broom from Oman
			AF361018	Ethiopian *Gliricidia* little leaf phytoplasma
X-disease group (16SrIII)	To be described as '*Ca.* Phytoplasma pruni'*	Suggested name†	L04682	Western X-disease mycoplasma-like organism
			X76430	*Mollicutes* (from *V. myrtillus*)
			AF373106	*Cirsium* white leaf phytoplasma rrnB
			AF373105	*Cirsium* white leaf phytoplasma rrnA
			AF370120	Delion virescence phytoplasma rrnB
			AF370119	Delion virescence phytoplasma rrnA
			AF495657	Chinaberry yellows phytoplasma
			AF177383	Soybean veinal necrosis phytoplasma
			AF060875	Virginia grapevine yellows phytoplasma VGYIII
			AF175304	Clover yellow edge phytoplasma
			AF056094	Sugarcane yellows phytoplasma type I strain ScYP I-Barb
			AF056095	Sugarcane yellows phytoplasma type I strain ScYP I-Afr
			AF147706	Chayote witches'-broom phytoplasma ChWBIII (Ch10)

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Phylogenetic group	'*Candidatus* Phytoplasma' species	Reference of the species description paper	GenBank accession no.	Database entry description
			AF147707	Chayote witches'-broom phytoplasma ChWBIII (Mor5)
			AF173558	Clover yellow edge phytoplasma
			AF236121	Peach rosette phytoplasma
			AF236122	Little peach phytoplasma
			AF189288	Clover yellow edge phytoplasma
			AF236123	Red suture phytoplasma
			AF190223	Poinsettia branch-inducing phytoplasma
			AF190226	Walnut witches'-broom phytoplasma rrnA
			AF190227	Walnut witches'-broom phytoplasma rrnB
			AF190228	*Spiraea* stunt phytoplasma
			AF244363	Black locust witches'-broom phytoplasma
			L33733	Canadian peach X mycoplasma-like organism
			D12580	Group II phytoplasma
			X77482	Mycoplasma-like organism (Italian clover phyllody)
			L33766	Clover yellow edge mycoplasma-like organism
			AF302841	Black raspberry witches'-broom phytoplasma clone BRWB7
			AF274876	Strawberry leafy fruit phytoplasma
Coconut lethal yellowing group (16SrIV)	To be described as '*Ca.* Phytoplasma palmae'*	Suggested name†	U18747	Coconut lethal yellowing phytoplasma
			AF237615	*Carludovica palmata* leaf yellowing phytoplasma
			AF434989	Texas Phoenix palm phytoplasma
			U18753	Yucatan coconut lethal decline phytoplasma
			AF361020	Florida Panus decline phytoplasma
	To be described as '*Ca.* Phytoplasma cocostanzaniae'*	Suggested name‡	X80117	*Phytoplasma* sp. strain LD
	To be described as '*Ca.* Phytoplasma cocosnigeriae'*	Suggested name‡	Y14175	*Phytoplasma* sp. strain LDN
			Y13912	*Phytoplasma* sp. strain LDG
	'*Ca.* Phytoplasma castaneae'	Jung *et al.* (2002)	AB054986	'*Candidatus* Phytoplasma castaneae'
Elm yellows group (16SrV)	'*Ca.* Phytoplasma ziziphi'	Jung *et al.* (2003a)	AY072722	*Ziziphus* jujube witches'-broom phytoplasma
			AF279272	*Ziziphus* jujube witches'-broom phytoplasma
			AF305240	*Ziziphus* jujube witches'-broom phytoplasma
	To be described as '*Ca.* Phytoplasma vitis'*	Suggested name†	AF176319	Flavescence dorée phytoplasma
			X76560	Mycoplasma (MLO; FD) transm. from *V. vinifera* to *V. faba*
	'*Ca.* Phytoplasma ulmi'	Lee *et al.* (2004b)	AF122910	Elm yellows phytoplasma strain EY1

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Phylogenetic group	'*Candidatus* Phytoplasma' species	Reference of the species description paper	GenBank accession no.	Database entry description
			AF122912	Phytoplasma HD1
			AF122911	Elm yellows phytoplasma strain WVEY
			AF189214	Elm yellows phytoplasma
			L33763	Elm yellows mycoplasma-like organism (rDNA)
			Y16395	*Rubus* stunt phytoplasma
			X68376	Mycoplasma-like organism (substrain ULW)
			AF305198	Virginia creeper phytoplasma
			AY028789	Alder yellows phytoplasma
			Y16387	Alder yellows phytoplasma
Clover proliferation group (16SrVI)	'*Ca.* Phytoplasma trifolii'	Hiruki & Wang (2004)	AY390261	'*Ca.* Phytoplasma trifolii'
			L33761	Clover proliferation mycoplasma-like organism (rDNA)
			AF036354	*Fragaria multicipita* phytoplasma
			AF190224	*Fragaria multicipita* phytoplasma rrnA
			AF190225	*Fragaria multicipita* phytoplasma rrnB
			X83431	*Mollicutes* sp. from *S. melongena*
			AF228052	Brinjal little leaf phytoplasma
			AF228053	Periwinkle little leaf phytoplasma
			AF409069	Clover proliferation phytoplasma strain EY-IL 2
			AF409070	Clover proliferation phytoplasma strain EY-IL 1
Ash yellows group (16SrVII)	'*Ca.* Phytoplasma fraxini'	Griffiths *et al.* (1999)	AF092209	Ash yellows phytoplasma
			AF105316	Ash yellows phytoplasma strain AshY5
			AF105315	Ash yellows phytoplasma strain AshY3
			AF105317	Ash yellows phytoplasma strain LWB3
			AF189215	Ash yellows phytoplasma
			X68339	Mycoplasma-like organism (substrain ASHY)
			L33759	Ash yellows mycoplasma-like organism (rDNA)
Loofah witches'-broom group (16SrVIII)	To be described as '*Ca.* Phytoplasma luffae'*	Suggested name†	AF086621	Loofah witches'-broom phytoplasma
			AF353090	Loofah witches'-broom phytoplasma str. LfWB clone1 rrnB
			AF248956	Loofah witches'-broom phytoplasma
			L33764	Loofah witches'-broom mycoplasma-like organism
Pigeon pea witches'-broom group (16SrIX)	'*Ca.* Phytoplasma phoenicium'	Verdin *et al.* (2003)	AF515636	'*Candidatus* Phytoplasma phoenicium'
			AF515637	'*Candidatus* Phytoplasma phoenicium'
			AF248957	Pigeon pea witches'-broom phytoplasma
			AF455038	Almond witches'-broom phytoplasma strain AlmWB3
			AF455041	Almond witches'-broom phytoplasma strain AlmWB-N1

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Table 1. cont.

Phylogenetic group	'*Candidatus* Phytoplasma' species	Reference of the species description paper	GenBank accession no.	Database entry description
			AF455040	Almond witches'-broom phytoplasma strain AlmWB-P1
			AF390136	Almond witches'- broom phytoplasma strain AlmWB1
			AF390137	Almond witches'- broom phytoplasma strain AlmWB2
			AF455039	Almond witches'-broom phytoplasma strain AlmWB4
			L33735	Pigeon pea witches'-broom mycoplasma-like organism
			Y18052	*Knautia arvensis* phyllody phytoplasma 23S
			Y16389	*Picris echioides* yellows phytoplasma
			U18763	Caribbean PPWB mycoplasma-like organism
			AF361017	Honduran *Gliricidia* little leaf phytoplasma
			AF361019	Florida *Rhynchosia* little leaf phytoplasma
Apple proliferation group (16SrX)	'*Ca.* Phytoplasma mali'	Seemüller & Schneider (2004)	AJ542541	Apple proliferation phytoplasma AP15
			AJ542542	Apple proliferation phytoplasma AP1/93
			AF248958	Apple proliferation phytoplasma
			X68375	Mycoplasma-like organism (substrain AT)
			X72206	Apple proliferation MLO
	'*Ca.* Phytoplasma pyri'	Seemüller & Schneider (2004)	AJ542543	Pear decline phytoplasma PD1
			X76425	*Mollicutes*
			Y16392	Pear decline phytoplasma
			Y16394	Peach yellow leafroll phytoplasma
	'*Ca.* Phytoplasma prunorum'	Seemüller & Schneider (2004)	AJ542544	European stone fruit yellows phytoplasma ESFY-G1
			AJ542545	European stone fruit yellows phytoplasma ESFY-G2
			AY029540	European stone fruit yellows phytoplasma
			X77372	Mycoplasma-like organism (plum leptonecrosis)
			X68374	Mycoplasma-like organism (substrain PPER)
			Y11933	*Phytoplasma* sp.
	'*Ca.* Phytoplasma spartii'	Marcone *et al.* (2004a)	X92869	*Phytoplasma* sp.
	'*Ca.* Phytoplasma rhamni'	Marcone *et al.* (2004a)	X76431	*Mollicutes* (from *R. frangula*)
	'*Ca.* Phytoplasma allocasuarinae'	Marcone *et al.* (2004a)	AY135523	'*Allocasuarina muelleriana*' phytoplasma
Rice yellow dwarf group (16SrXI)	'*Ca.* Phytoplasma oryzae'	Jung *et al.* (2003b)	D12581	Group III phytoplasma
Stolbur group (16SrXII)	'*Ca.* Phytoplasma australiense'	Davis *et al.* (1997)	L76865	Australian grapevine yellows phytoplasma
			U43570	*Phormium* yellow leaf phytoplasma rrnB
			U43569	*Phormium* yellow leaf phytoplasma rrnA
			AJ243045	Strawberry lethal yellows phytoplasma
			AJ243044	Strawberry green petal phytoplasma
	To be described as '*Ca.* Phytoplasma solani'*	Suggested name†	AF248959	Stolbur phytoplasma
			X76427	*Mollicutes* (from *C. anuum* to *C. roseus*)
			X76428	*Mollicutes* (from *V. vinifera*)

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Table 1. cont.

Phylogenetic group	'*Candidatus* Phytoplasma' species	Reference of the species description paper	GenBank accession no.	Database entry description
BGWL group (16SrXIV)	'*Ca.* Phytoplasma cynodontis'	Marcone *et al.* (2004b)	AJ550984	Bermuda grass white leaf phytoplasma
			AJ550985	Bermuda grass white leaf phytoplasma
			AJ550986	Bermuda grass white leaf phytoplasma
			AF248961	Bermuda grass white leaf phytoplasma
			AF509321	*Cynodon* white leaf phytoplasma
'*Ca.* Phytoplasma brasiliense' group (16SrXV)	'*Ca.* Phytoplasma brasiliense'	Montano *et al.* (2001)	AF147708	*Hibiscus* witches'-broom phytoplasma strain HibWB26
Other phytoplasmas not related to the above *Candidatus* species according to 16S rRNA gene sequences:				
Mexican periwinkle virescence group (16SrXIII)	No name suggested		AF248960	Mexican periwinkle virescence phytoplasma
			U96614	*Phytoplasma* sp. STRAWB1
Not assigned	No name suggested		AF495882	Chinaberry yellows phytoplasma strain CbY1
			AJ289195	Vigna little leaf phytoplasma
			Y16391	Bindweed yellows phytoplasma
			AJ310849	*Phytoplasma* sp. PinP
			Y17055	*Phytoplasma* sp. (strain StLL)
			AJ289192	*Stylosanthes* little leaf phytoplasma
			X76429	*Mollicutes* (from *C. roseus*)
			X76432	*Mollicutes* (from *S. officinarum*)
			X83438	*Mollicutes* sp. 16S rRNA gene and tRNA-Ile
			AF509324	Sorghum grassy shoot phytoplasma variant I
			AF509325	Sorghum grassy shoot phytoplasma variant II
			Y15865	*Phytoplasma* sp. (strain GaLL)

*According to Rule 28b of the Bacteriological Code, this is an incidental citation and does not constitute prior citation.

†Name proposed by the IRPCM Phytoplasma Working Team at the X International Congress of the International Organization of Mycoplasmology, Bordeaux, 1994.

‡Name proposed by the IRPCM Phytoplasma/Spiroplasma Working Team at the XIV International Congress of the International Organization of Mycoplasmology, Vienna, 2002.

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(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 PCR-based assay.

(d) The rank of subspecies should not be used.

(e) The reference strain should be made available to the scientific community from the authors of the *Candidatus* species description paper and it should be deposited (unless *in vitro* micropropagation proves impossible) in the micropropagated collection of Dr Assunta Bertaccini, DiSTA, Patologia Vegetale, Università di Bologna, Italy.

(f) Manuscripts that describe a novel '*Ca.* Phytoplasma' species should preferably be submitted to the *International Journal of Systematic and Evolutionary Microbiology* (IJSEM).

(g) The abbreviation for *Candidatus* is *Ca.* (e.g. '*Ca.* Phytoplasma japonicum' stands for '*Candidatus* Phytoplasma japonicum').

It is recommended that future descriptions of '*Ca.* Phytoplasma' species follow the above rules. In order to provide a standard reference for the calculation of 16S rRNA gene sequence similarities between potential novel isolates and currently described '*Ca.* Phytoplasma' species, a reference sequence alignment is available from TreeBase under accession no. S1048-1788 (see Methods). For direct comparison and reference, strains that have been used to define each '*Ca.* Phytoplasma' species can be obtained from the collection of A. Bertaccini (see address above). For phytoplasmas that cannot be maintained in *in vitro* propagation collections, reference DNA (total DNA extracted from an infected plant) should be obtained from the corresponding author of the species description papers.

Table 1 reports a list of taxa that belong to '*Ca.* Phytoplasma', identified as described above, and a list of 16S rRNA gene sequences of related strains. The listed taxa either have already been described individually in past issues of IJSEM or are described in accompanying papers in this issue or in forthcoming papers; the reader is referred to the original papers for their detailed descriptions (Zreik *et al.*, 1995; Davis *et al.*, 1997; White *et al.*, 1998; Griffiths *et al.*, 1999; Sawayanagi *et al.*, 1999; Montano *et al.*, 2001; Jung *et al.*, 2002, 2003a, b; Hiruki & Wang, 2004; Lee *et al.*, 2004a, b; Marcone *et al.*, 2004a, b; Seemüller & Schneider, 2004). The taxon '*Ca.* Phytoplasma australasia' is not retained, as its 16S rRNA gene sequence is 99·5 % similar to that of '*Ca.* Phytoplasma aurantifolia' and there is no evidence that conditions (i) and (iii) of rule (c) above are satisfied.

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