

'*Candidatus Phytoplasma solani*', a novel taxon associated with stolbur- and bois noir-related diseases of plants

Fabio Quaglino,¹ Yan Zhao,² Paola Casati,¹ Daniela Bulgari,¹ Piero Attilio Bianco,¹ Wei Wei^{2,3} and Robert Edward Davis²

Correspondence
Robert Edward Davis
robert.davis@ars.usda.gov

¹Dipartimento di Scienze Agrarie e Ambientali - Produzione, Territorio, Agroenergia, Università degli Studi, via Celoria 2, 20133 Milan, Italy

²Molecular Plant Pathology Laboratory, USDA-Agricultural Research Service, Beltsville, MD 20705, USA

³Institute for Bioscience and Biotechnology Research, University of Maryland, College park, MD 20742, USA

Phytoplasmas classified in group 16SrXII infect a wide range of plants and are transmitted by polyphagous planthoppers of the family Cixiidae. Based on 16S rRNA gene sequence identity and biological properties, group 16SrXII encompasses several species, including '*Candidatus Phytoplasma australiense*', '*Candidatus Phytoplasma japonicum*' and '*Candidatus Phytoplasma fragariae*'. Other group 16SrXII phytoplasma strains are associated with stolbur disease in wild and cultivated herbaceous and woody plants and with bois noir disease in grapevines (*Vitis vinifera* L.). Such latter strains have been informally proposed to represent a separate species, '*Candidatus Phytoplasma solani*', but a formal description of this taxon has not previously been published. In the present work, stolbur disease strain STOL11 (STOL) was distinguished from reference strains of previously described species of the '*Candidatus Phytoplasma*' genus based on 16S rRNA gene sequence similarity and a unique signature sequence in the 16S rRNA gene. Other stolbur- and bois noir-associated ('*Ca. Phytoplasma solani*') strains shared >99% 16S rRNA gene sequence similarity with strain STOL11 and contained the signature sequence. '*Ca. Phytoplasma solani*' is the only phytoplasma known to be transmitted by *Hyalesthes obsoletus*. Insect vectorship and molecular characteristics are consistent with the concept that diverse '*Ca. Phytoplasma solani*' strains share common properties and represent an ecologically distinct gene pool. Phylogenetic analyses of 16S rRNA, *tuf*, *secY* and *rplV-rpsC* gene sequences supported this view and yielded congruent trees in which '*Ca. Phytoplasma solani*' strains formed, within the group 16SrXII clade, a monophyletic subclade that was most closely related to, but distinct from, that of '*Ca. Phytoplasma australiense*'-related strains. Based on distinct molecular and biological properties, stolbur- and bois noir-associated strains are proposed to represent a novel species level taxon, '*Ca. Phytoplasma solani*'; STOL11 is designated the reference strain.

Phytoplasmas (formerly mycoplasma-like organisms, MLOs) are pleomorphic, cell-wall-less bacteria of the class *Mollicutes* that exist as obligate trans-kingdom parasites inhabiting phloem sieve cells in plants and diverse cell

types in insect vectors. In their descent from free-living, walled bacteria in the *Bacillus/Clostridium* group, evolutionary genome reduction resulted in loss or disruption of genes encoding various metabolic pathways (Davis *et al.*, 2003; Oshima *et al.*, 2004; Weisburg *et al.*, 1989), and as a consequence, researchers have been unable to obtain phytoplasmas in axenic culture. Phenotypic characters used in descriptions of species in the class *Mollicutes* have therefore been inaccessible for phytoplasmas. A phytoplasma group classification system has been established on the basis of RFLP analysis of 16S rRNA gene sequences and a provisional species taxonomy of the '*Candidatus Phytoplasma*' genus based on 16S rRNA gene phylogeny

Abbreviations: DSB, distinguishing sequence block; SNP, single nucleotide polymorphism.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *tuf*, *secY* and ribosomal protein (*rp*) gene sequences of '*Candidatus Phytoplasma solani*' STOL11^R are AF248959, JQ797670, JQ797668, and JQ797662, respectively.

Three supplementary figures are available with the online version of this paper.

Table 1. 'Candidatus *Phytoplasma solani*'-related strains used for sequence alignments and phylogenetic analyses

Nucleotide sequences produced in the present work are related to strain names and accession numbers in bold type. All nucleotide sequences of the *tuf*, *secY* and *rplV-rpsC* genes were analysed and shown in the figures.

Strain	Disease description	Reference	Associated host	Geographical origin	GenBank accession no.			
					16S rRNA	<i>tuf</i>	<i>secY</i>	<i>rplV-rpsC</i>
STOL11 (STOL)	Stolbur	Davis & Dally, 2001	<i>Capsicum annuum</i>	Serbia	AF248959*†	JQ797670	JQ797668	JQ797662
Rus-PPT92	Potato purple top	Girsova <i>et al.</i> , 2008	<i>Solanum tuberosum</i>	Russia	EU344884†	–	–	–
Rus-PPT94	Potato purple top	Girsova <i>et al.</i> , 2008	<i>Solanum tuberosum</i>	Russia	EU344885‡	–	–	–
Rus-PPT97	Potato purple top	Girsova <i>et al.</i> , 2008	<i>Solanum tuberosum</i>	Russia	EU344886‡	–	–	–
Rus-PPT100	Potato purple top	Girsova <i>et al.</i> , 2008	<i>Solanum tuberosum</i>	Russia	EU344887†	–	–	–
Rus-PPT109	Potato purple top	Girsova <i>et al.</i> , 2008	<i>Solanum tuberosum</i>	Russia	EU344888‡	–	–	–
Rus-PPT111	Potato purple top	Girsova <i>et al.</i> , 2008	<i>Solanum tuberosum</i>	Russia	EU344889‡	–	–	–
Rus-PPT124	Potato purple top	Girsova <i>et al.</i> , 2008	<i>Solanum tuberosum</i>	Russia	EU344890*	–	–	–
IPPT§	Potato purple top	Hosseini <i>et al.</i> , 2011	<i>Solanum tuberosum</i>	Iran	EU661607*†	–	–	–
TPSP§	Potato stolbur	Eroglu <i>et al.</i> , 2010	<i>Solanum tuberosum</i>	Turkey	HM485579*	–	–	–
STOLC	Stolbur	Minucci & Boccardo, 1997	<i>Lycopersicon esculentum</i>	France	–	–	AM992083	–
LG	Stolbur	Minucci & Boccardo, 1997	<i>Lycopersicon esculentum</i>	France	–	–	AM992092	–
T2_92	Stolbur	Minucci & Boccardo, 1997	<i>Lycopersicon esculentum</i>	Italy	–	–	AM992088	–
T2_56	Stolbur	Minucci & Boccardo, 1997	<i>Lycopersicon esculentum</i>	Italy	–	–	AM992087	–
PTV	Stolbur	Minucci & Boccardo, 1997	<i>Lycopersicon esculentum</i>	Italy	–	–	–	EF193364
4MN	Bois noir	Radonjić <i>et al.</i> , 2009	<i>Vitis vinifera</i> L.	Montenegro	–	FJ441241	–	–
6MN	Bois noir	Radonjić <i>et al.</i> , 2009	<i>Vitis vinifera</i> L.	Montenegro	–	FJ441242	–	–
R47/5	Bois noir	Berger <i>et al.</i> , 2009	<i>Vitis vinifera</i> L.	Italy	–	FJ394552	–	–
R49/15	Bois noir	Berger <i>et al.</i> , 2009	<i>Vitis vinifera</i> L.	Italy	–	FJ394551	–	–
CH1	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy	–	–	AM992089	–
BN-Op30	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Lombardy)	EU836652*	–	–	–
BN-Op121	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Lombardy)	EU836651†	–	–	–
BN-Op261	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Lombardy)	EU836650*	–	–	–
BN-Fc13	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Lombardy)	EU836649‡	–	–	–
BN-Fc12	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Lombardy)	EU836648‡	–	–	–
BN-Fc3	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Lombardy)	EU836647*†	–	–	–
BN-Op125	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Lombardy)	EU836646‡	–	–	–
BN-Fc11	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Lombardy)	EU836645‡	–	–	–
BN-Fc213	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Lombardy)	EU836644*	–	–	–
BN-Op224	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Lombardy)	EU836658*	–	–	–
BN-Op123	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Lombardy)	EU836657*†	–	–	–
BN-Fc24	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Lombardy)	EU836655‡	–	–	–
BN-Fc89	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Lombardy)	EU836654‡	–	–	–
BN-Fc55	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Lombardy)	EU836653‡	–	–	–
BN-Ma202	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Marche)	FJ409899†	GU220564	–	–

Table 1. cont.

Strain	Disease description	Reference	Associated host	Geographical origin	GenBank accession no.			
					16S rRNA	<i>tuf</i>	<i>secY</i>	<i>rplV-rpsC</i>
BN-Ma198	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Marche)	FJ409898*	–	–	–
BN-Ma182	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Marche)	FJ409895‡	–	–	–
BN-Ab175	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Abruzzi)	FJ409894*	–	–	–
BN-Ab170	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Abruzzi)	FJ409893†	GU220561	–	–
BN-Ab164	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Abruzzi)	FJ409892‡	–	–	–
BN-Ab150	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Abruzzi)	FJ409891‡	–	–	–
BN-Si238	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Sicily)	–	GU220565	–	–
BN-Fc6	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Lombardy)	–	GU220558	JQ797669	JQ797663
BN-Fc15	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Lombardy)	–	GU220559	–	–
BN-Fc76	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Lombardy)	–	GU220560	–	–
BN-Op37	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Lombardy)	–	GU220562	–	–
BN-Op40	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Lombardy)	–	GU220563	–	–
BN-Vr277	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Veneto)	–	–	–	JQ797666
BN-Vr279	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Veneto)	–	–	–	JQ797665
BN-Vr283	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Veneto)	–	–	–	JQ797667
BN-Vr285	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy (Veneto)	–	–	–	JQ797664
134_04	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy/France	EU014780*†	–	–	–
104_04	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy/France	EU014779‡	–	–	–
93_04	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy/France	EU014778‡	–	–	–
92_04	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy/France	EU014777†	–	–	–
85_04	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy/France	EU014776‡	–	–	–
425_05	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy/France	EU010010*	–	–	–
415_05	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy/France	EU010009‡	–	–	–
409_05	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy/France	EU010008‡	–	–	–
400_05	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy/France	EU010007†	–	–	–
391_05	Bois noir	Belli <i>et al.</i> , 2010	<i>Vitis vinifera</i> L.	Italy/France	EU010006‡	–	–	–
2642BN	Bois noir	Torres <i>et al.</i> , 2005	<i>Vitis vinifera</i> L.	Spain	AJ964960*†	–	–	–
VK	Bois noir	Seemüller <i>et al.</i> , 1994	<i>Vitis vinifera</i> L.	Germany	X76428*†	–	–	–
19-25	Bois noir	Seemüller <i>et al.</i> , 1994	<i>Vitis vinifera</i> L.	Germany	–	–	AM992094	–
GGY	Bois noir	Seemüller <i>et al.</i> , 1994	<i>Vitis vinifera</i> L.	Germany	–	–	AM992093	–
IL14-6	Bois noir	Šeruga <i>et al.</i> , 2000	<i>Vitis vinifera</i> L.	Croatia	–	EU717134	–	–
IL14-5	Bois noir	Šeruga <i>et al.</i> , 2000	<i>Vitis vinifera</i> L.	Croatia	–	EU717133	–	–
IL14-4	Bois noir	Šeruga <i>et al.</i> , 2000	<i>Vitis vinifera</i> L.	Croatia	–	EU717132	–	–
IL13-4	Bois noir	Šeruga <i>et al.</i> , 2000	<i>Vitis vinifera</i> L.	Croatia	–	EU717131	–	–
IL11-3	Bois noir	Šeruga <i>et al.</i> , 2000	<i>Vitis vinifera</i> L.	Croatia	–	EU717130	–	–
DRC5	Bois noir	Šeruga <i>et al.</i> , 2000	<i>Vitis vinifera</i> L.	Croatia	–	EU717129	–	–
DRC3	Bois noir	Šeruga <i>et al.</i> , 2000	<i>Vitis vinifera</i> L.	Croatia	–	EU717128	–	–
DRC1	Bois noir	Šeruga <i>et al.</i> , 2000	<i>Vitis vinifera</i> L.	Croatia	–	EU717127	–	–

Table 1. cont.

Strain	Disease description	Reference	Associated host	Geographical origin	GenBank accession no.			
					16S rRNA	<i>tuf</i>	<i>secY</i>	<i>rplV-rpsC</i>
JSK10-2	Bois noir	Šeruga <i>et al.</i> , 2000	<i>Vitis vinifera</i> L.	Croatia	–	EU717126	–	–
IL11-4	Bois noir	Šeruga <i>et al.</i> , 2000	<i>Vitis vinifera</i> L.	Croatia	–	EU717124	–	–
IL13-3	Bois noir	Šeruga <i>et al.</i> , 2000	<i>Vitis vinifera</i> L.	Croatia	–	EU717122	–	–
IL14-3	Bois noir	Šeruga <i>et al.</i> , 2000	<i>Vitis vinifera</i> L.	Croatia	–	EU717123	–	–
GHRAN08	Bois noir	Karimi <i>et al.</i> , 2009	<i>Vitis vinifera</i> L.	Iran	GQ403235*†	–	–	–
25-17-14	Red clover dwarf	Fránová <i>et al.</i> , 2009	<i>Trifolium pratense</i>	Czech Republic	EU814637†	EU814638	–	–
26-16-33	Red clover dwarf	Fránová <i>et al.</i> , 2009	<i>Trifolium pratense</i>	Czech Republic	EU814644‡	EU814643	–	–
25-5-48	Red clover dwarf	Fránová <i>et al.</i> , 2009	<i>Trifolium pratense</i>	Czech Republic	EU814640‡	EU814641	–	–
1-38-40	Red clover dwarf	Fránová <i>et al.</i> , 2009	<i>Trifolium pratense</i>	Czech Republic	–	EU552455	–	–
3-21-36	Red clover dwarf	Fránová <i>et al.</i> , 2009	<i>Trifolium pratense</i>	Czech Republic	EU814645*	–	–	–
M1V	Strawberry yellows	Terlizzi <i>et al.</i> , 2006	<i>Fragaria x ananassa</i>	Italy	–	DQ418457	–	–
R1V	Strawberry yellows	Terlizzi <i>et al.</i> , 2006	<i>Fragaria x ananassa</i>	Italy	–	DQ418460	–	–
F11	Strawberry yellows	Terlizzi <i>et al.</i> , 2006	<i>Fragaria x ananassa</i>	Italy	–	DQ418459	–	–
F9	Strawberry yellows	Terlizzi <i>et al.</i> , 2006	<i>Fragaria x ananassa</i>	Italy	–	DQ418456	–	–
F5	Strawberry yellows	Terlizzi <i>et al.</i> , 2006	<i>Fragaria x ananassa</i>	Italy	–	DQ418458	–	–
2005/02	Corn reddening	Duduk & Bertaccini, 2006	<i>Zea mays</i>	Serbia	DQ222972*†	–	–	–
RHOD-CZ	Rhododendron leaf malformation	Mertelik <i>et al.</i> , 2004	<i>Rhododendron</i> sp.	Czech Republic	DQ160244*†	–	–	–
RHOD1-CZ	Rhododendron leaf malformation	Mertelik <i>et al.</i> , 2004	<i>Rhododendron</i> sp.	Czech Republic	DQ160245*	–	–	–
G64	Stolbur	Zwolinska <i>et al.</i> , 2012	<i>Pisum sativum</i>	Poland	GU060495*†	–	–	–
Charente-1	–	–	<i>Hyalesthes obsoletus</i>	France	–	–	AM992084	–
Charente-2	–	–	<i>Hyalesthes obsoletus</i>	France	–	–	AM992085	–
PO	–	–	<i>Hyalesthes obsoletus</i>	France	–	–	AM992082	–
P7	Stolbur	Choueiri <i>et al.</i> , 2002	<i>Catharanthus roseus</i> L.	Lebanon	–	–	AM992091	–
BN-Ma192	Stolbur	Marcone <i>et al.</i> , 1997	<i>Convolvulus arvensis</i>	Italy	FJ409896*	–	–	–
BN-Ma193	Stolbur	Marcone <i>et al.</i> , 1997	<i>Convolvulus arvensis</i>	Italy	FJ409897*	–	–	–
YLS1	Stolbur	Arocha <i>et al.</i> , 2005	<i>Macroptilium lathyroides</i>	Cuba	AY725233*†	–	–	–
Red-pepper	Stolbur	Cimerman <i>et al.</i> , 2009	<i>Capsicum annuum</i>	France	–	–	AM992086	–
Moliere	Stolbur	Cimerman <i>et al.</i> , 2009	<i>Prunus mahaleb</i>	France	–	–	AM992090	–
Solmal	Stolbur	Iriti <i>et al.</i> , 2008	<i>Solanum glaucophyllum</i>	Italy	–	EF153635	–	–
AppST§	Stolbur	Duduk <i>et al.</i> , 2010	<i>Malus domestica</i>	China	FJ685752*†	–	–	–
AprST§	Stolbur	Duduk <i>et al.</i> , 2010	<i>Prunus armeniaca</i>	China	FJ685753*	–	–	–
PJ15 I	Stolbur	Zirak <i>et al.</i> , 2009	<i>Prunus mume</i>	Iran	FJ409624*	–	–	–
PP49	Stolbur	Zirak <i>et al.</i> , 2010	<i>Prunus persica</i>	Iran	FJ204394*	–	–	–

*Representative 16S rRNA gene nucleotide sequences selected for building the phylogenetic tree shown in Fig. 2(a).

†Representative 16S rRNA gene nucleotide sequences selected for illustrating signature sequences and DSBs in Fig. S1.

‡16S rRNA gene nucleotide sequences included in alignments and phylogenetic analyses, but not shown in order to improve clarity of the figures.

§IPPT, Iranian potato purple top phytoplasma; TPSP, Turkish potato stolbur phytoplasma; AppST, Apple stolbur phytoplasma; AprST, Apricot stolbur phytoplasma.

Table 2. Additional group 16SrXII 'Candidatus Phytoplasma' strains used for phylogenetic analyses

All nucleotide sequences of the *tuf*, *secY* and *rplV-rpsC* genes were analysed and shown in the figures.

Strain	Disease description	Reference	Associated host	Geographical origin	GenBank accession no.			
					16S rRNA	<i>tuf</i>	<i>secY</i>	<i>rplV-rpsC</i>
AUSGY*	Australian grapevine yellows	Davis <i>et al.</i> , 1997	<i>Vitis vinifera</i> L.	Australia	L76865†‡	–	–	–
AGY	Australian grapevine yellows	Padovan <i>et al.</i> , 1995	<i>Vitis vinifera</i> L.	Australia	X95706†	–	–	–
CaPaus*	Cottonbush reduced yellows leaves	Streten & Gibb, 2005	<i>Gomphocarpus physocarpus</i>	Australia	NC_010544‡	NC_010544	NC_010544	NC_010544
CBRYL	Cottonbush reduced yellows leaves	Streten & Gibb, 2005	<i>Gomphocarpus physocarpus</i>	Australia	–	AY303554	–	AY303559
CBWB	Cottonbush witches'-broom	Streten & Gibb, 2005	<i>Gomphocarpus physocarpus</i>	Australia	–	AY303553	–	AY303558
SGP	Strawberry green petal	Padovan <i>et al.</i> , 2000	<i>Fragaria x ananassa</i>	Australia	AJ243044†‡	AY303571	–	AY303570
SLY	Strawberry lethal yellows	Padovan <i>et al.</i> , 2000	<i>Fragaria x ananassa</i>	Australia	AJ243045†‡	–	–	–
SLY1	Strawberry lethal yellows	Padovan <i>et al.</i> , 2000	<i>Fragaria x ananassa</i>	Australia	–	AY303556	–	AY303561
SLY2	Strawberry lethal yellows	Padovan <i>et al.</i> , 2000	<i>Fragaria x ananassa</i>	Australia	–	AY303552	–	AY303562
StrVirP*	Strawberry virescence	Davis <i>et al.</i> , 2006	<i>Fragaria x ananassa</i>	Australia	AY377868†‡	–	–	–
NZSb7	Strawberry lethal yellows	Andersen <i>et al.</i> , 1998	<i>Fragaria x ananassa</i>	New Zealand	–	DQ094778	–	–
NZSb9	Strawberry lethal yellows	Andersen <i>et al.</i> , 1998	<i>Fragaria x ananassa</i>	New Zealand	–	DQ094779	–	–
NZSb11	Strawberry lethal yellows	Andersen <i>et al.</i> , 1998	<i>Fragaria x ananassa</i>	New Zealand	–	DQ096804	–	–
PYL	Phormium yellow leaf	Liefting <i>et al.</i> , 1997	<i>Phormium tenax</i>	New Zealand	U43570†‡	–	–	–
NZPh1	Phormium yellow leaf	Liefting <i>et al.</i> , 1997	<i>Phormium tenax</i>	New Zealand	–	DQ094772	–	–
NZPh3	Phormium yellow leaf	Liefting <i>et al.</i> , 1997	<i>Phormium tenax</i>	New Zealand	–	DQ094773	–	–
NZPh5	Phormium yellow leaf	Liefting <i>et al.</i> , 1997	<i>Phormium tenax</i>	New Zealand	–	DQ094774	–	–
NZPh6	Phormium yellow leaf	Liefting <i>et al.</i> , 1997	<i>Phormium tenax</i>	New Zealand	–	DQ094775	–	–
NZPh7	Phormium yellow leaf	Liefting <i>et al.</i> , 1997	<i>Phormium tenax</i>	New Zealand	–	DQ094776	–	–
NZPh9	Phormium yellow leaf	Liefting <i>et al.</i> , 1997	<i>Phormium tenax</i>	New Zealand	–	DQ094777	–	–
NZ09156	Potato purple leaf	Liefting <i>et al.</i> , 2009	<i>Solanum tuberosum</i>	New Zealand	FJ943262†‡	FJ943263	–	–
PpDB	Papaya dieback	White <i>et al.</i> , 1998	<i>Carica papaya</i>	Australia	Y10095†‡	–	–	–
PDB	Papaya dieback	White <i>et al.</i> , 1998	<i>Carica papaya</i>	Australia	–	Y18215	–	–
PYCL	Pumpkin yellow leaf curl	Streten & Gibb, 2005	<i>Cucurbita</i> sp.	Australia	–	AY303555	–	AY303560
NZCt4	Cordyline sudden decline	Streten & Gibb, 2005	<i>Cordyline australis</i>	New Zealand	–	DQ094780	–	–
Sr Isidro	Peach yellow leaf roll-like	Jones <i>et al.</i> , 2005	<i>Prunus persicae</i>	Bolivia	AY725212†	–	–	–
CaPjap*	Japanese hydrangea phylloidy	Sawayanagi <i>et al.</i> , 1999	<i>Hydrangea macrophylla</i>	Japan	AB010425†‡	–	–	–
CaPfra*	Strawberry yellows	Valiunas <i>et al.</i> , 2006	<i>Fragaria x ananassa</i>	Lithuania	DQ086423†‡	–	–	–

*AUSGY, 'Ca. Phytoplasma australiense' reference strain AUSGY; CaPaus, 'Ca. Phytoplasma australiense'; StrVirP, strawberry virescence phytoplasma; CaPjap, 'Ca. Phytoplasma japonicum'; CaPfra, 'Ca. Phytoplasma fragariae'.

†Representative 16S rRNA gene nucleotide sequences selected for building the phylogenetic tree shown in Fig. 2(a).

‡Representative 16S rRNA gene nucleotide sequences selected for illustrating signature sequences and DSBs in Fig. S1.

Table 3. Insect vectors of 'Candidatus Phytoplasma' species of group 16SrXII (stolbur group) and their distribution

Phytoplasma*	Disease	Vector	Distribution	Reference
CaPsol	Bois noir	<i>Hyalesthes obsoletus</i> Signoret	Europe	Maixner, 1994
	Bois noir	<i>Anaceratagallia ribauti</i> Ossiannilsson	Austria	Riedle-Bauer <i>et al.</i> , 2008
	Stolbur	<i>Hyalesthes obsoletus</i> Signoret	Europe	Brckak, 1979
	Stolbur	<i>Pentastiridius beieri</i> Wagner	France	Gatineau <i>et al.</i> , 2001
	Stolbur	<i>Macrostes quadripunctulatus</i>	Europe	Battle <i>et al.</i> , 2008
	Maize redness	<i>Reptalus panzeri</i> Low	Serbia	Jović <i>et al.</i> , 2007
CaPaus	Phormium yellow leaf	<i>Oliarius atkinsoni</i> Meyers	New Zealand	Liefting <i>et al.</i> , 1997
CaPjap	Japanese hydrangea phyllody	Not determined	Japan	Sawayanagi <i>et al.</i> , 1999
CaPfra	Strawberry yellows	Not determined	Lithuania	Valiunas <i>et al.</i> , 2006

*CaPsol, 'Ca. Phytoplasma solani'; CaPaus, 'Ca. Phytoplasma australiense'; CaPjap, 'Ca. Phytoplasma japonicum'; CaPfra, 'Ca. Phytoplasma fragariae'.

and biological characteristics has been adopted (IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group, 2004; Lee *et al.*, 2000; Seemüller *et al.*, 1994). To facilitate description of closely related species of phytoplasma, Davis *et al.* (2013) recently proposed that analyses of non-rRNA genes be employed to distinguish closely related species, and that non-rRNA gene sequences be incorporated as part of amended and future formal descriptions of species of the 'Ca. Phytoplasma' genus. Phytoplasmas classified in 16S rRNA gene RFLP group 16SrXII infect a wide range of wild and cultivated plants worldwide and are transmitted by polyphagous planthoppers of the family *Cixiidae*. Three species of the 'Ca. Phytoplasma' genus have thus far been formally described within group 16SrXII: (i) 'Candidatus Phytoplasma australiense', infecting grapevine and other plant hosts in Australia and New Zealand (Davis *et al.*, 1997); (ii) 'Candidatus Phytoplasma japonicum', infecting Japanese hydrangea in Japan (Sawayanagi *et al.*, 1999); and (iii) 'Candidatus Phytoplasma fragariae', infecting strawberry in Europe (Valiunas *et al.*, 2006). Phytoplasma strains associated with stolbur (STOL) and stolbur-related diseases in wild and cultivated herbaceous and woody plants and with bois noir (BN) disease in cultivated grapevines, hereinafter referred to as stolbur strains, have been informally proposed to represent a separate species, 'Ca. Phytoplasma solani' (IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group, 2004), but a formal description of this taxon has not been previously published. In the present communication, on the basis of nucleotide sequence and phylogenetic analyses of 16S rRNA, *tuf*, *secY* and *rplV-rpsC* genes and assessment of biological properties, stolbur phytoplasma strains are described as representing a distinct species-level lineage and novel taxon, 'Candidatus Phytoplasma solani'.

DNA fragments of the *tuf*, *secY* and *rplV-rpsC* genes were PCR-amplified from strain STOL (STOL11), which was originally from a diseased plant of *Capsicum annuum* in Serbia (Seemüller *et al.*, 1994) and had been maintained by grafting in plants of Madagascar periwinkle [*Catharanthus*

roseus (L.) G. Don]. DNA fragments of the *rplV-rpsC* locus were amplified from bois noir-associated phytoplasma strains BN-Fc6, BN-Vr277, BN-Vr279, BN-Vr283 and BN-Vr285, and a DNA fragment of *secY* was amplified from phytoplasma strain BN-Fc6. Strains from grapevine plants showing typical symptoms of bois noir grapevine yellows disease in the Lombardy (north-western Italy) and Veneto (north-eastern Italy) regions were identified by PCR-RFLP analyses of the 16S rRNA gene, as previously described (Quaglino *et al.*, 2009). PCR conditions and primers were as described by Gundersen *et al.* (1996), Langer & Maixner (2004), Martini *et al.* (2007) and Lee *et al.* (2010). PCR-products were cloned in plasmid vector pCRII-TOPO (Invitrogen) and propagated in *Escherichia coli*. Both strands of cloned inserts were sequenced to achieve at least 4 × coverage per base position. DNA sequencing was performed by a commercial sequencing service (Primm, Milan, Italy). The obtained nucleotide sequences were deposited in the GenBank database under accession numbers given in Table 1. Nucleotide sequences of 16S rRNA, *tuf*, *secY* and *rplV-rpsC* genes from diverse group 16SrXII strains, and from reference strains of formally described species of the 'Ca. Phytoplasma' genus, were retrieved from the GenBank database; accessions containing two or more consecutive undetermined nucleotides were excluded from analyses (Tables 1 and 2).

Amino acid sequences of Elongation Factor Tu, ribosomal proteins L22 and S3, and protein translocase subunit SecY, deduced from nucleotide sequences of *tuf*, *rplV-rpsC* and *secY*, respectively, using the ExPasy (<http://www.expasy.org/>) software (Gasteiger *et al.*, 2003), were aligned by the software BioEdit version 7.0.5 (<http://www.mbio.ncsu.edu/bioedit/bioedit.html>).

Data on plant hosts, insect vectors and geographical distribution of strains of 'Ca. Phytoplasma solani' and of previously described species of phytoplasma ('Ca. Phytoplasma australiense', 'Ca. Phytoplasma fragariae' and 'Ca. Phytoplasma japonicum') classified in group 16SrXII were collected and compiled (Tables 1–3). The biological features of the phytoplasmas were compared in

Table 4. Per cent 16S rRNA gene sequence similarity of ‘*Candidatus Phytoplasma solani*’ strain STOL11 with reference strains of members of the ‘*Candidatus Phytoplasma*’ genus previously described

Recently, ‘*Candidatus Phytoplasma balanitae*’ was proposed, but the 16S rRNA gene sequence was not available for comparison at the time of writing. Since, according to the article, this phytoplasma species is most closely related to ‘*Ca. Phytoplasma ziziphi*’ and ‘*Ca. Phytoplasma ulmi*’, unavailability of that sequence should not affect the delineation of ‘*Candidatus Phytoplasma solani*’.

‘ <i>Candidatus Phytoplasma</i> ’ species (GenBank no.)	Segment size (bp)	Similarity (%)
‘ <i>Ca. Phytoplasma australiense</i> ’ (L76865)	1375	97.6
‘ <i>Ca. Phytoplasma graminis</i> ’ (AY725228)	1521	96.1
‘ <i>Ca. Phytoplasma caricae</i> ’ (AY725234)	1516	95.5
‘ <i>Ca. Phytoplasma fragariae</i> ’ (DQ086423)	1333	97.1
‘ <i>Ca. Phytoplasma americanum</i> ’ (DQ174122)	1503	96.8
‘ <i>Ca. Phytoplasma asteris</i> ’ (M30790)	1535	96
‘ <i>Ca. Phytoplasma japonicum</i> ’ (AB010425)	1521	96
‘ <i>Ca. Phytoplasma convolvuli</i> ’ (JN833705)	1496	96.7
‘ <i>Ca. Phytoplasma costaricanum</i> ’ (HQ225630)	1537	94.8
‘ <i>Ca. Phytoplasma lycopersici</i> ’ (EF199549)	1519	93.5
‘ <i>Ca. Phytoplasma spartii</i> ’ (X92869)	1522	91.4
‘ <i>Ca. Phytoplasma prunorum</i> ’ (AJ542544)	1521	91.5
‘ <i>Ca. Phytoplasma tamaricis</i> ’ (FJ432664)	1514	91.3
‘ <i>Ca. Phytoplasma pyri</i> ’ (AJ542543)	1516	91.3
‘ <i>Ca. Phytoplasma mali</i> ’ (AJ542541)	1521	91.1
‘ <i>Ca. Phytoplasma allocasuarinae</i> ’ (AY135523)	1152	91.2
‘ <i>Ca. Phytoplasma rhamni</i> ’ (X76431)	1437	90.1
‘ <i>Ca. Phytoplasma phoenicium</i> ’ (AF515636)	1502	88.5
‘ <i>Ca. Phytoplasma aurantifolia</i> ’ (U15442)	1513	88.8
‘ <i>Ca. Phytoplasma pruni</i> ’ <i>rrnA</i> (JQ044393)	1517	89.3
‘ <i>Ca. Phytoplasma pruni</i> ’ <i>rrnB</i> (JQ044392)	1517	89.3
‘ <i>Ca. Phytoplasma malaysianum</i> ’ (EU371934)	1523	88.8
‘ <i>Ca. Phytoplasma brasiliense</i> ’ (AF147708)	1526	88.9
‘ <i>Ca. Phytoplasma omanense</i> ’ (EF666051)	1424	88.9
‘ <i>Ca. Phytoplasma australasia</i> ’ (Y10097)	1521	88.9
‘ <i>Ca. Phytoplasma cynodontis</i> ’ (AJ550984)	1508	88.8
‘ <i>Ca. Phytoplasma fraxini</i> ’ (AF092209)	1496	89.2
‘ <i>Ca. Phytoplasma pini</i> ’ (AJ632155)	1531	88.8
‘ <i>Ca. Phytoplasma trifolii</i> ’ (AY390261)	1531	88.9
‘ <i>Ca. Phytoplasma oryzae</i> ’ (AB052873)	1360	88.6
‘ <i>Ca. Phytoplasma sudamericanum</i> ’ (GU292081)	1375	88.8
‘ <i>Ca. Phytoplasma ulmi</i> ’ (AY197655)	1527	88.4
‘ <i>Ca. Phytoplasma rubi</i> ’ (AY197648)	1529	88.3
‘ <i>Ca. Phytoplasma ziziphi</i> ’ (AB052876)	1367	87.9
‘ <i>Ca. Phytoplasma castaneae</i> ’ (AB054986)	1522	87.7

order to determine whether stolbur and bois noir phytoplasma strains clearly formed an ecologically distinct population.

‘*Ca. Phytoplasma solani*’, a distinct gene pool

According to guidelines by the IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group (2004), a phytoplasma strain can be described as a novel species if its 16S rRNA gene sequence shares <97.5% similarity with that of any previously described species of the ‘*Ca. Phytoplasma*’ genus. Strain STOL11, selected as the potential reference strain of the putative species ‘*Ca. Phytoplasma solani*’, shares <97.5% 16S rRNA gene sequence similarity with all previously described species

of the ‘*Ca. Phytoplasma*’ genus except ‘*Ca. Phytoplasma australiense*’ (Table 4).

Since, of all previously described ‘*Ca. Phytoplasma*’ species, only ‘*Ca. Phytoplasma australiense*’ shared >97.5% 16S rRNA gene sequence similarity with strain STOL11, we focused on distinctions between STOL11 and ‘*Ca. Phytoplasma australiense*’ and other members of group 16SrXII. We first determined whether intra-species 16S rRNA gene sequence similarity was consistently greater than inter-species sequence similarity between ‘*Ca. Phytoplasma solani*’ and ‘*Ca. Phytoplasma australiense*’. The results showed that phytoplasma strains associated with bois noir- and stolbur-related diseases (‘*Ca. Phytoplasma solani*’ strains) shared >99% 16S rRNA gene

Table 5. Group 16SrXII phytoplasma strains: their hosts, geographical origins and 16S rRNA gene sequence similarities with 'Ca. Phytoplasma solani' strain STOL11 and 'Ca. Phytoplasma australiense' strain AUSGY

Strain	Host	Origin	Acc. no.	Size (bp)	% similarity with:	
					STOL11	AUSGY
'Ca. Phytoplasma solani'						
Rus-PPT92	<i>Solanum tuberosum</i>	Russia	EU344884	1502	99.6	97.9
Rus-PPT100	<i>Solanum tuberosum</i>	Russia	EU344887	1502	99.6	97.9
IPPT	<i>Solanum tuberosum</i>	Iran	EU661607	1243	99.6	98.2
BN-Op121	<i>Vitis vinifera</i> L.	Italy	EU836651	1243	99.4	97.9
BN-Fc3	<i>Vitis vinifera</i> L.	Italy	EU836647	1243	99.3	97.9
BN-Op123	<i>Vitis vinifera</i> L.	Italy	EU836657	1243	99.2	97.8
BN-Ma202	<i>Vitis vinifera</i> L.	Italy	FJ409899	1243	99.5	98
BN-Ab170	<i>Vitis vinifera</i> L.	Italy	FJ409893	1243	99.3	97.9
134_04	<i>Vitis vinifera</i> L.	Italy/France	EU014780	1465	99.5	97.7
92_04	<i>Vitis vinifera</i> L.	Italy/France	EU014777	1465	99.3	97.5
400_05	<i>Vitis vinifera</i> L.	Italy/France	EU010007	1465	99.5	97.7
2642BN	<i>Vitis vinifera</i> L.	Spain	AJ964960	1522	99.6	97.8
VK	<i>Vitis vinifera</i> L.	Germany	X76428	1501	99.4	97.7
GrIRAN08	<i>Vitis vinifera</i> L.	Iran	GQ403235	1455	99.4	97.6
25-17-14	<i>Trifolium pratense</i>	Czech Republic	EU814637	1458	99.7	97.9
2005/02	<i>Zea mays</i>	Serbia	DQ222972	1525	99.2	97.4
RHOD-CZ	<i>Rhododendron</i> sp.	Czech Republic	DQ160244	1243	99.1	97.6
G64	<i>Pisum sativum</i>	Poland	GU060495	1243	99.6	98.2
YLS1	<i>Macroptilium lathyroides</i>	Cuba	AY725233	1509	99.4	97.6
Red-pepper	<i>Capsicum annuum</i>	Serbia	X76427	1494	99.2	97.5
AppST	<i>Malus domestica</i>	China	FJ685752	1358	99.6	97.8
Other group 16SrXII phytoplasma						
CaPaus	<i>Gomphocarpus physocarpus</i>	Australia	NC_010544	1533	97.7	99.9
SGP	<i>Fragaria x ananassa</i>	Australia	AJ243044	1489	97.5	99.7
SLY	<i>Fragaria x ananassa</i>	Australia	AJ243045	1478	97.6	99.7
StrVirP	<i>Fragaria x ananassa</i>	Australia	AY377868	1478	96.7	99
PYL	<i>Phormium tenax</i>	New Zealand	U43570	1483	97.8	99.7
NZ09156	<i>Solanum tuberosum</i>	New Zealand	FJ943262	1525	97.9	99.8
PpDB	<i>Carica papaya</i>	Australia	Y10095	1521	97.9	99.8

sequence identity with strain STOL11 and shared <98.2% 16S rRNA gene sequence identity with 'Ca. Phytoplasma australiense' reference strain AUSGY. On the other hand, diverse 'Ca. Phytoplasma australiense'-related strains shared >99% 16S rRNA gene sequence identity with strain AUSGY and ≤97.9% 16S rRNA gene sequence identity with strain STOL11 (Table 5). Thus, 16S rRNA gene intra-species sequence similarity was significantly and consistently greater than inter-species similarity.

Multiple 16S rRNA gene sequence alignments identified STOL11-unique signature sequences in nucleotide sequence AF248959 (Fig. 1). The sequence (5'-ATTTTT-AAAAGACCTAGCAATAGGTATGCTTAG-3') at nucleotide positions 189–221 differed by at least one base from comparable regions in 16S rRNA genes of all previously described species, and differed at three base positions from the comparable region in the 16S rRNA gene of 'Ca. Phytoplasma australiense' AUSGY.

In addition, the combination of two other sequence regions in the 16S rRNA gene distinguished strain STOL11 from all

previously named species; we designated these sequence regions as distinguishing sequence blocks (DSBs). DSB1, the nucleotide sequence at base positions 452–480 (5'-ATGGTGGAAAAACCATTATGACGGTACCT-3'), is shared by strain STOL11 and reference strains of 'Ca. Phytoplasma australiense', 'Ca. Phytoplasma fragariae' and 'Candidatus Phytoplasma americanum' (Davis *et al.*, 1997; Lee *et al.*, 2006; Valiunas *et al.*, 2006), but DSB1 distinguished STOL11 from all other named species. DSB2, the nucleotide sequence at base positions 602–627 (5'-GCAACGCTCAACGTTGTGATGCTATA-3'), is shared by strain STOL11 and the reference strains of 'Candidatus Phytoplasma caricae' and 'Candidatus Phytoplasma graminis' (Arocha *et al.*, 2005), but DSB2 distinguishes STOL11 from other species. Based on combined consideration of signature and DSB sequences, strain STOL11 is distinguished from reference strains of previously reported phytoplasma species by at least four single nucleotide polymorphisms (SNPs) across the three nucleotide sequence regions noted here (Fig. 1).

In previous work, 16S rRNA gene signature sequences have been employed as molecular markers for separating

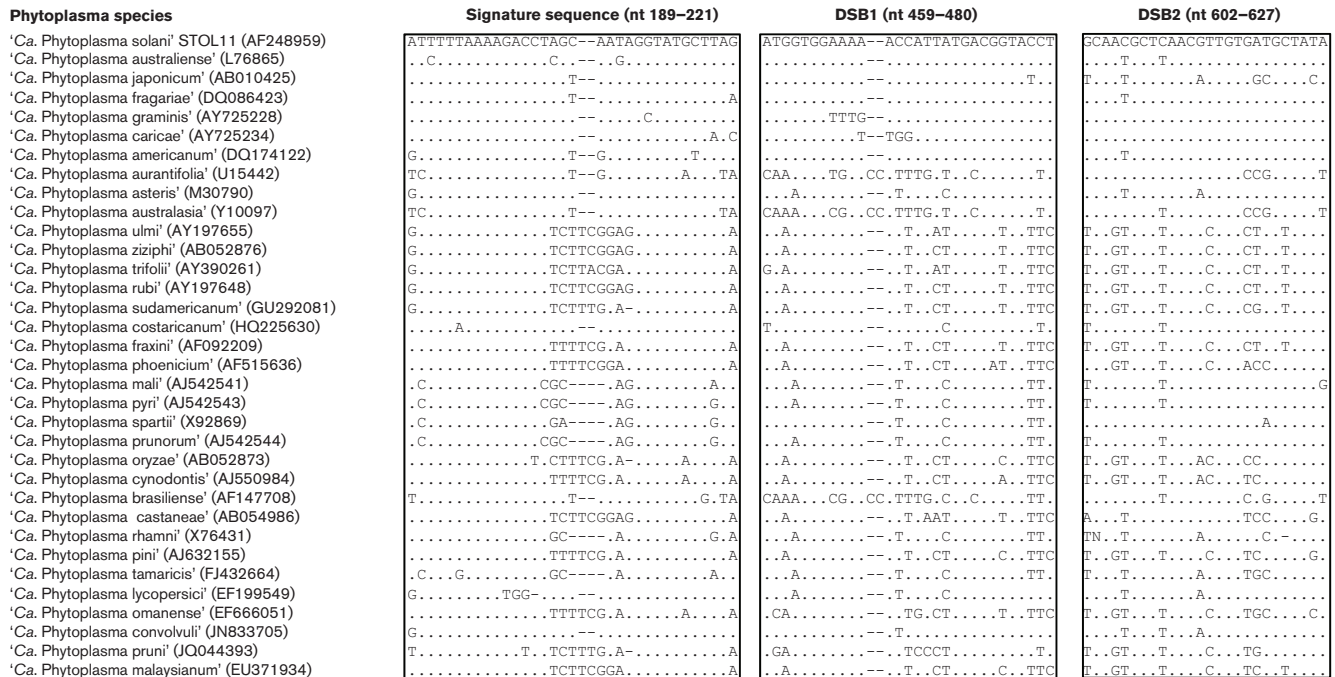


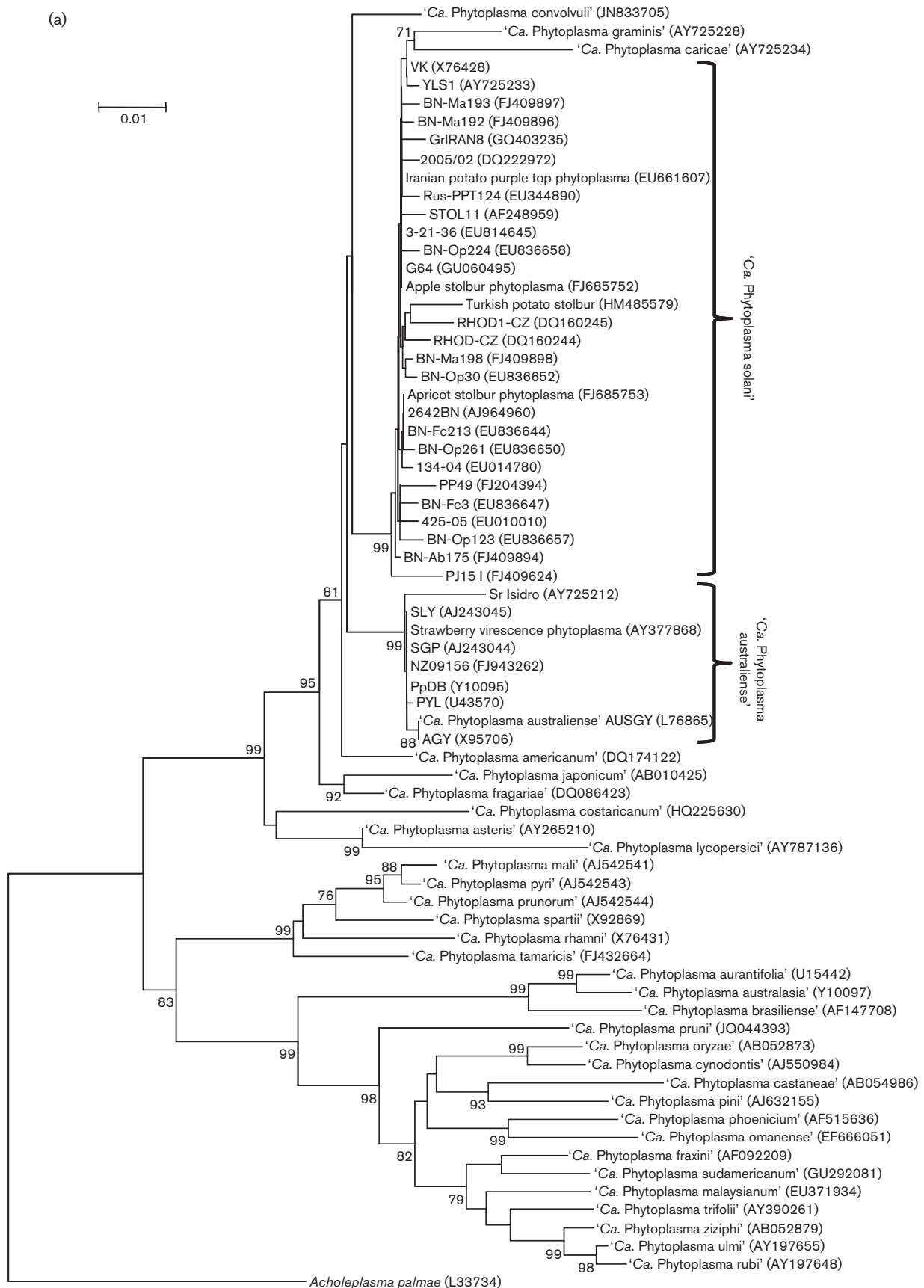
Fig. 1. Alignment of 16S rRNA gene nucleotide sequences, from reference strains of previously described species of the genus 'Ca. Phytoplasma', trimmed to regions equivalent to signature sequence and DSBs of 'Ca. Phytoplasma solani' strain STOL11. Sequence alignments were carried out using the 'ClustalW Multiple Alignment' application of BioEdit version 7.0.5 (<http://www.mbio.ncsu.edu/bioedit/bioedit.html>) by selecting the 'do complete alignment' option with default parameters. The alignments were searched for sequences distinguishing strain STOL11 and other stolbur (stolbur and bois noir) strains from reference strains of previously described species of the genus 'Ca. Phytoplasma'. nt, Nucleotide positions in GenBank accession AF248959.

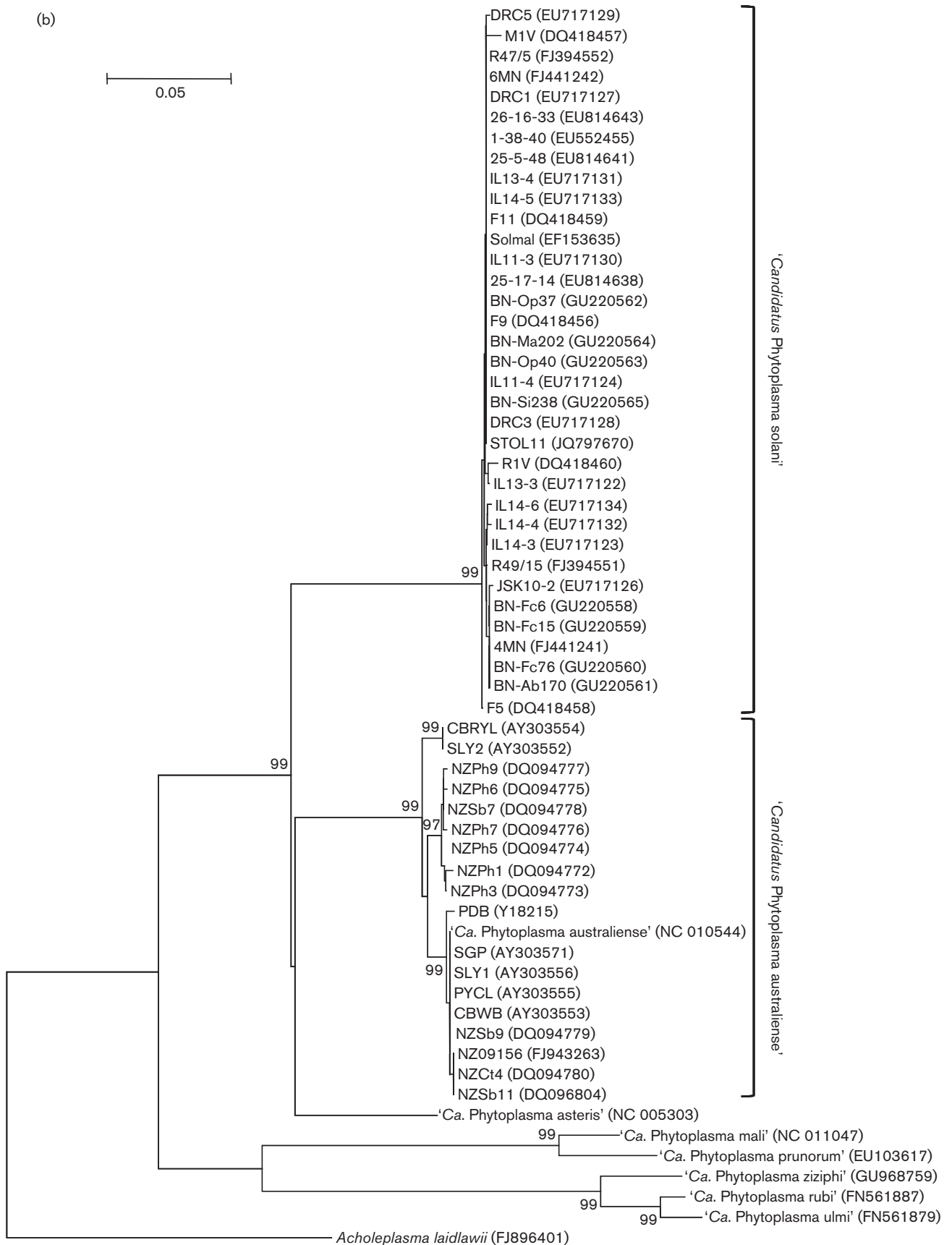
biologically distinct 'Ca. Phytoplasma' species that shared >97.5% 16S rRNA gene sequence similarity (Seemüller & Schneider, 2004). In the absence of distinctive 16S rRNA gene signature sequences, molecular markers within less conserved genes (*rplV*–*rpsC*, *secY*, *tuf*) have been used (Malembic-Maher *et al.*, 2011). In addition to distinctive SNPs localized within the STOL11-specific signature sequence for distinguishing 'Ca. Phytoplasma solani' strains from closely related phytoplasmas, we introduce the synergistic utilization of signatures and DSBs. This combined use of 16S rRNA gene signature and DSB sequences aids the definition of 'Ca. Phytoplasma solani' and facilitates assignment of strains to the species. Specifically, we suggest that analyses of 16S rRNA gene sequence similarity and comparative analyses of signature sequence and DSBs should be combined for determining whether a given strain should be termed a 'Ca. Phytoplasma solani' strain. In our view, a strain of 'Ca. Phytoplasma solani' should (i) share >99% sequence similarity with a minimum of 1.2 kb within the 16S rRNA gene of reference strain STOL11 and (ii) contain the identical STOL11 signature (unique) 16S rRNA gene sequence, and (iii) both DSB sequences noted for reference strain STOL11 with a tolerance of a single nucleotide difference in no more than one of the sequences (Figs 1 and

S1, available in IJSEM Online). Strains that do not fulfil either criterion (ii) or criterion (iii) are considered 'Ca. Phytoplasma solani'-related strains, even if they fulfil criterion (i).

Phylogenetic analyses of 16S rRNA genes yielded a tree in which STOL11, and diverse bois noir and stolbur phytoplasma strains, formed a distinct clade that apparently shared a common ancestor with 'Ca. Phytoplasma australiense' (Fig. 2a). Interestingly, 'Ca. Phytoplasma graminis' and 'Ca. Phytoplasma caricae' (Arocha *et al.*, 2005) clustered on long branches within the 'Ca. Phytoplasma solani' clade, possibly indicating environment-influenced, divergent evolution of these three species from a common ancestor. Moreover, 'Candidatus Phytoplasma convolvuli' (Martini *et al.*, 2012) clustered closely to the 'Ca. Phytoplasma solani' clade. As 'Ca. Phytoplasma convolvuli' is specifically associated with *Convolvulus arvensis* in nature (Martini *et al.*, 2012) and 'Ca. Phytoplasma solani' is capable of infecting the same plant species (Marcone *et al.*, 1997), it would be interesting to learn whether the two species evolved from a common ancestor that was able to infect *C. arvensis*.

Although strain STOL11 shared only 97.6% 16S rRNA gene sequence similarity with strain AUSGY, reference





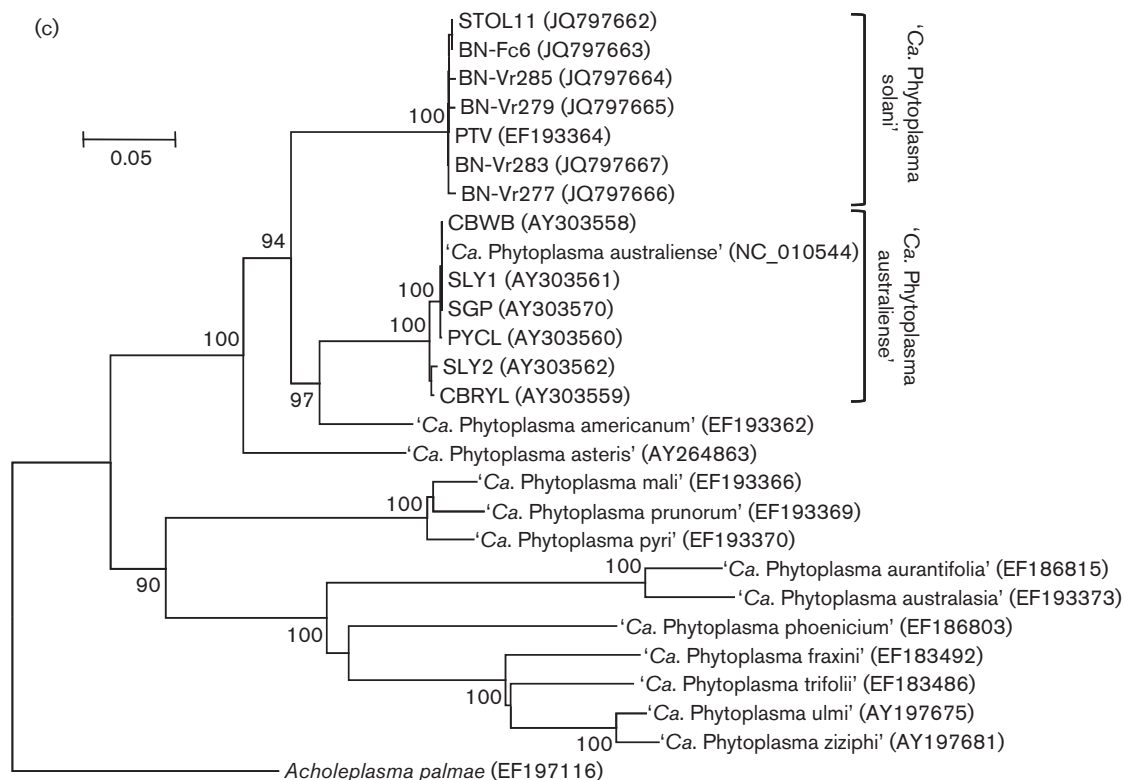


Fig. 2. Phylogenetic trees inferred from analyses of nucleotide sequences of 16S rRNA (a), *tuf* (b) and *rpIV-rpsC* (c) genes. Minimum evolution analysis was carried out using the neighbour-joining method with the software MEGA4 (Kumar *et al.*, 2008). The reliability of the analyses was subjected to a bootstrap test with 1000 replicates; bootstrap values lower than 90 are not shown. Strains of '*Ca. Phytoplasma solani*' and '*Ca. Phytoplasma australiense*' formed two distinct subclades. Nucleotide sequence accession numbers are given in the trees. *Acholeplasma palmae* and *Acholeplasma laidlawii* were used for rooting the trees. Bars, 0.01 (a), 0.05 (b) and 0.05 (c) substitutions per nucleotide position.

strain of '*Ca. Phytoplasma australiense*' (Table 4), 16S rRNA gene sequence similarity alone may be insufficient for delineating and describing STOL11 as a representative of a novel species. However, according to IRPCM guidelines (IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group, 2004), phytoplasmas sharing >97.5% 16S rRNA gene nucleotide sequence similarity can be described as separate species if they are clearly distinguished by evident molecular diversity and ecological niche. These latter criteria were previously employed for delineating '*Candidatus Phytoplasma ulmi*', '*Candidatus Phytoplasma rubi*', '*Candidatus Phytoplasma mali*', '*Candidatus Phytoplasma prunorum*' and '*Candidatus Phytoplasma pyri*' (Lee *et al.*, 2004; Malembic-Maher *et al.*, 2011; Seemüller & Schneider, 2004).

Unique vectorship of '*Ca. Phytoplasma solani*' strains

We examined whether '*Ca. Phytoplasma solani*' strains are characterized by unique ecological and/or biological properties. Focus was on comparisons with '*Ca. Phytoplasma australiense*', the only described species sharing >97.5%

sequence similarity of the 16S rRNA gene, and other species classified within group 16SrXII. Our findings affirm that species of the '*Ca. Phytoplasma*' genus within group 16SrXII possess distinct biological properties. In Australia and New Zealand, '*Ca. Phytoplasma australiense*' strains are associated with Australian grapevine yellows disease (Padovan *et al.*, 1995) and with other diseases characterized by witches'-broom growths, leaf yellowing and purpling, leaf rolling or curling, flower petal greening (virescence), mild or lethal decline, and/or phyllody on plants of various families (Table 2). A member of the Cixiidae, *Oliarius atkinsoni* Meyers, was described as the insect vector of the '*Ca. Phytoplasma australiense*' strain associated with phormium yellow leaf disease in New Zealand (Liefting *et al.*, 1997) (Table 3). Recently, strains of '*Ca. Phytoplasma australiense*' were reported outside the Oceanic continent in association with peach yellow leaf roll-like disease in Bolivia (Jones *et al.*, 2005) and with Nivun Haamir dieback in Israel (Gera *et al.*, 2005). '*Ca. Phytoplasma japonicum*' is associated with Japanese hydrangea phyllody disease in Japan (Sawayanagi *et al.*, 1999); '*Ca. Phytoplasma fragariae*' is the presumptive aetiological agent of strawberry yellows disease in Lithuania

(Valiunas *et al.*, 2006) (Table 2). Insect vectors of the latter two species of the 'Ca. Phytoplasma' genus have not yet been reported (Table 3). In Europe and the Mediterranean basin, strains of 'Ca. Phytoplasma solani' are associated with bois noir disease of grapevine, with stolbur disease in wild and cultivated herbaceous and woody plants, and with yellowing, reddening, decline, dwarfism, leaf malformation and degeneration diseases of other plants (Table 1). Several insects of the families Cixiidae and Cicadellidae have been reported as vectors of strains of 'Ca. Phytoplasma solani' (Table 3). *Hyalesthes obsoletus*, the most common vector, is not known to transmit any other phytoplasma, possibly indicating a long and intimate co-evolution of phytoplasma and vector, and a unique phytoplasma–vector association distinguishing 'Ca. Phytoplasma solani' from other species. This hypothesis is reinforced by the finding that strains of 'Ca. Phytoplasma solani' identified in *H. obsoletus* and in its preferred host plant *C. arvensis*, clustered together within the 'Ca. Phytoplasma solani' clade in phylogenetic trees built on the basis of 16S rRNA and *secY* gene sequences (Figs 2a and S2). In particular, the presence of identical *secY* gene signature sequences in 'Ca. Phytoplasma solani' strains, identified in *H. obsoletus* and its host plant (Fig. 3) is consistent with an intimate symbiotic relationship between the insect vector and the 'Ca. Phytoplasma solani' strains. Recent studies indicate that an insect's capability to transmit a phytoplasma is determined by specific binding of insect cytoskeleton microfilaments (Suzuki *et al.*, 2006) to phytoplasmal antigenic membrane proteins, transported by the Sec system (Kakizawa *et al.*, 2004), which includes the protein

SecY. Thus, the available data clearly indicate that a distinct ecological niche is occupied by 'Ca. Phytoplasma solani'.

Strains of 'Ca. Phytoplasma solani' distinguished by analysis of sequence variable genes

Since additional genes were proposed as criteria for future delineation of species (Davis *et al.*, 2013), further work was carried out that distinguished 'Ca. Phytoplasma solani' from other species on the basis of sequence analyses of *tuf*, *secY* and *rplV-rpsC* gene loci, as well as biological properties. Nucleotide sequence alignments revealed unique SNPs in the *tuf*, *secY* and *rplV-rpsC* genes of STOL11 and other 'Ca. Phytoplasma solani' strains. Within the *tuf* gene sequence, 29 SNPs are unique in STOL11 and the other strains of 'Ca. Phytoplasma solani' (Fig. S3a). Within the *secY* gene sequence, 52 SNPs distinguished the strains of 'Ca. Phytoplasma solani' from other phytoplasmas (Fig. S3b). Within the *rplV-rpsC* gene locus, 31 SNPs distinguished the strains of 'Ca. Phytoplasma solani' from other phytoplasma species (Fig. S3c). Moreover, strains of 'Ca. Phytoplasma solani' and 'Ca. Phytoplasma australiense' shared *tuf*, *secY* and *rplV-rpsC* gene inter-species sequence similarities of 81.7–87.1%, 54.5–75.3% and 74.7–82.1%, respectively. By contrast, strains of 'Ca. Phytoplasma solani' shared *tuf*, *secY* and *rplV-rpsC* gene sequence intra-species similarities of 98.7–100%, 99.1–100% and 97.4–99.7%, respectively; whereas, strains of 'Ca. Phytoplasma australiense' shared *tuf*, *secY* and *rplV-rpsC* intra-species sequence similarities of 93–100%, 100% and 89.3–100%, respectively. Thus, 'Ca. Phytoplasma

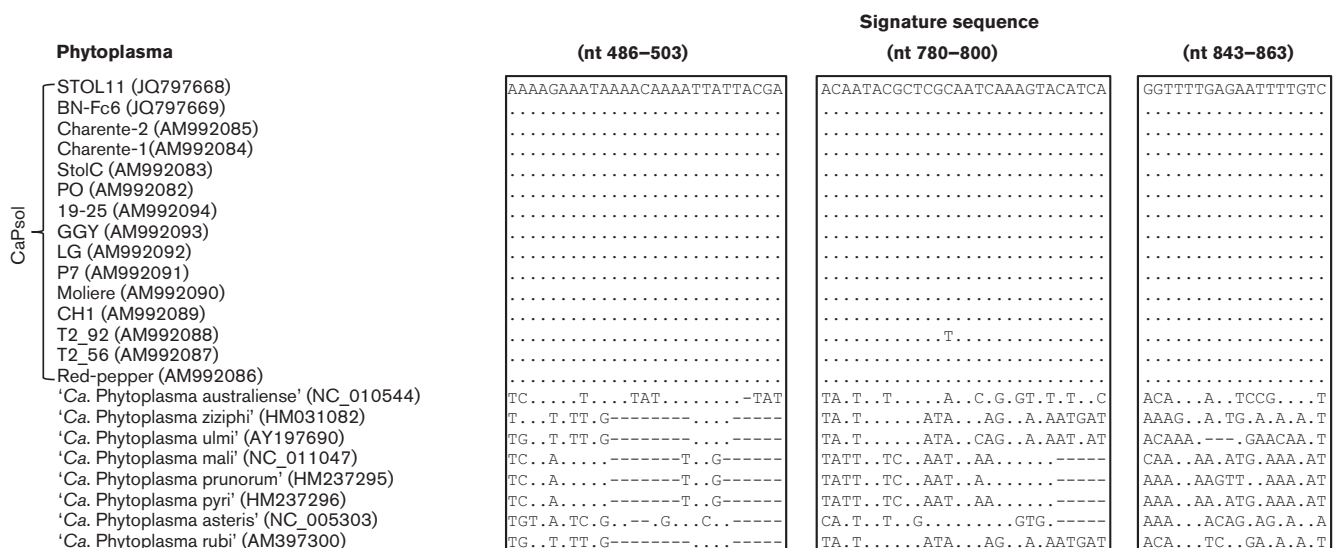


Fig. 3. Alignment of *secY* nucleotide sequences, from 'Ca. Phytoplasma solani' (CaPso) strains and from selected other species, trimmed to regions equivalent to three signature sequence regions in the *secY* gene of 'Ca. Phytoplasma solani' strain STOL11. Sequences were aligned using the software BioEdit version 7.0.5. Aligned nucleotide sequences were trimmed to a common, shared DNA fragment of approximately 900 nt. Accessions with a shorter sequence were not used for analysis. nt, Nucleotide positions in GenBank accession JQ797668.

solani' and 'Ca. *Phytoplasma australiense*' *tuf*, *secY* and *rplV-rpsC* intra-species sequence similarity was remarkably and consistently greater than inter-species similarity.

Alignment of multiple *secY* gene sequences identified three STOL11-unique signature sequences in sequence JQ797668 at positions 486–503, 780–800 and 843–863 from the start codon (Fig. 3). Such unique (signature) sequences differed by numerous bases from comparable regions in the *secY* gene of 'Ca. *Phytoplasma australiense*' (NC_010544) and of previously described species. These STOL11-unique sequences were shared by all strains of 'Ca. *Phytoplasma solani*' (Fig. 3).

Phylogenetic analyses of *tuf*, *rplV-rpsC* and *secY* gene sequences yielded trees in which strains of 'Ca. *Phytoplasma solani*' consistently formed a distinct clade (Figs 2b, c and S3). Clustering of strains of 'Ca. *Phytoplasma solani*' was confirmed by phylogenetic analyses of deduced amino acid sequences of EF-Tu, rplL22-rpS3 and SecY proteins (data not shown). In summary, the distinct molecular characteristics and unique vectorship support recognition of 'Ca. *Phytoplasma solani*' as a novel species in the genus 'Ca. *Phytoplasma*'.

Description of 'Candidatus *Phytoplasma solani*'

'Candidatus *Phytoplasma solani*' (so.la'ni. N.L. gen. n. solani of *Solanum* the scientific name of a genus including solanaceous plants; the epithet referring to plant hosts).

Reference strain is STOL11^R.

[(*Mollicutes*) NC; NA; O, wall less; NAS (GenBank accession number AF248959), oligonucleotide sequence of unique region of the 16S rRNA gene (5'-ATTTT-TAAAAGACCTAGCAATAGGTATGCTTAG-3') nt 189–221, oligonucleotide sequences of DSBs of the 16S rRNA gene (5'-ATGGTGGAAAAACCATTATGACGGTACCT-3') nt 452–480 and (5'-GCAACGCTCAACGTTGTGA-TGCTATA-3') nt 602–627; oligonucleotide sequences of unique regions of the *secY* gene, GenBank no. JQ797668, (5'-TATGGCCTCAATGAGTAATAATT-3') nt 486–503, (5'-TTTCAAATGTAAATCCCAAT-3') nt 780–800 and (5'-AACTAATATTGACATCGTTAA-3') nt 843–863].

Note added in proof

While this communication was in press, the paper describing 'Ca. *Phytoplasma convolvuli*' was published [Martini *et al.* (2012). *Int J Syst Evol Microbiol* 62, 2910–2915]. The Martini *et al.* (2012) paper does not affect any of the conclusions in the present communication.

References

- Andersen, M. T., Longmore, J., Liefting, L. W., Wood, G. A., Sutherland, P. W., Beck, D. L. & Forster, R. L. S. (1998). Phormium yellow leaf phytoplasma is associated with strawberry lethal yellows disease in New Zealand. *Plant Disease* 82, 606–609.
- Arocha, Y., López, M., Piñol, B., Fernández, M., Picornell, B., Almeida, R., Palenzuela, I., Wilson, M. R. & Jones, P. (2005).

'Candidatus *Phytoplasma graminis*' and 'Candidatus *Phytoplasma caricae*', two novel phytoplasmas associated with diseases of sugarcane, weeds and papaya in Cuba. *Int J Syst Evol Microbiol* 55, 2451–2463.

Battle, A., Altabella, N., Sabate, J. & Lavina, A. (2008). Study of the transmission of stolbur phytoplasma to different crop species, by *Macrostelus quadripunctulatus*. *Ann Appl Biol* 152, 235–242.

Belli, G., Bianco, P. A. & Conti, M. (2010). Grapevine yellows in Italy: past, present and future. *J Plant Pathol* 92, 303–326.

Berger, J., Dalla Via, J. & Baric, S. (2009). Development of a TaqMan allelic discrimination assay for the distinction of two major subtypes of the grapevine yellows phytoplasma Bois noir. *Eur J Plant Pathol* 124, 521–526.

Brcajk, J. (1979). Leafhopper and planthopper vectors of plant disease agents in central and southern Europe. In *Leafhopper Vectors and Plant Disease Agents*, pp. 97–146. Edited by K. Maramorosch & K. F. Harris. London: Academic Press.

Choueiri, E., Jreijiri, F., El-Zammar, S., Verdin, E., Salar, P., Danet, J. L., Bove, J. & Garnier, M. (2002). First report of grapevine "bois noir" disease and a new phytoplasma infecting solanaceous plants in Lebanon. *Plant Disease* 86, 697.

Cimerman, A., Pacifico, D., Salar, P., Marzachi, C. & Foissac, X. (2009). Striking diversity of *vmp1*, a variable gene encoding a putative membrane protein of the stolbur phytoplasma. *Appl Environ Microbiol* 75, 2951–2957.

Davis, R. E. & Dally, E. L. (2001). Revised subgroup classification of group 16SrV phytoplasmas and placement of *Flavescence dorée*-associated phytoplasmas in two distinct subgroups. *Plant Disease* 85, 790–797.

Davis, R. E., Dally, E. L., Gundersen, D. E., Lee, I.-M. & Habili, N. (1997). 'Candidatus *Phytoplasma australiense*', a new phytoplasma taxon associated with Australian grapevine yellows. *Int J Syst Bacteriol* 47, 262–269.

Davis, R. E., Jomantiene, R., Zhao, Y. & Dally, E. L. (2003). Folate biosynthesis pseudogenes, PsifolP and PsifolK, and an O-sialoglycoprotein endopeptidase gene homolog in the phytoplasma genome. *DNA Cell Biol* 22, 697–706.

Davis, R. I., Jones, P., Holman, T. J., Halsey, K., Amice, R., Tupouniua, S. K. M. & Seth, M. (2006). Phytoplasma disease surveys in Tonga, New Caledonia and Vanuatu. *Australas Plant Pathol* 35, 335–340.

Davis, R. E., Zhao, Y., Dally, E., Lee, I.-M., Jomantiene, R. & Douglas, S. M. (2013). '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 Bacteriol* 63, 766–776.

Duduk, B. & Bertaccini, A. (2006). Corn with symptoms of reddening: new host of stolbur phytoplasma. *Plant Disease* 90, 1313–1319.

Duduk, B., Tian, J., Contaldo, N., Fan, X., Paltrinieri, S., Chen, Q., Zhao, Q. & Bertaccini, A. (2010). Occurrence of phytoplasmas related to stolbur and to 'Candidatus *Phytoplasma japonicum*' in woody host plants in China. *J Phytopathol* 158, 100–104.

Eroglu, S., Ozbek, H. & Sahin, F. (2010). First report of group 16SrXII phytoplasma causing stolbur disease in potato plants in the eastern and southern Anatolia regions of Turkey. *Plant Disease* 94, 1374.

Fránová, J., Navratil, M. & Jakesova, H. (2009). Molecular identification of stolbur phytoplasma associated with red clover dwarf disease symptoms. *J Phytopathol* 157, 502–506.

Gasteiger, E., Gattiker, A., Hoogland, C., Ivanyi, I., Appel, R. D. & Bairoch, A. (2003). ExPASy: The proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Res* 31, 3784–3788.

- Gatineau, F., Larrue, J., Clair, D., Lorton, F., Richard-Molard, M. & Boudon-Padieu, E. (2001). A new natural planthopper vector of stolbur phytoplasma in the genus *Pentastiridius* (Hemiptera: Cixiidae). *Eur J Plant Pathol* **107**, 263–271.
- Gera, A., Mawassi, M., Zeidan, M., Spiegel, S. & Bar-Joseph, M. (2005). An isolate of 'Candidatus Phytoplasma australiense' group associated with Nivun Haamir dieback disease of papaya in Israel. *Plant Pathol* **54**, 560.
- Girsova, N., Bottner, K. D., Mozhaeva, K. A., Kastalyeva, T. B., Owens, R. A. & Lee, I.-M. (2008). Molecular detection and identification of group 16SrI and 16SrXII phytoplasmas associated with diseased potatoes in Russia. *Plant Disease* **92**, 654.
- Gundersen, D. E. & Lee, I.-M. (1996). Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. *Phytopathol Mediterr* **35**, 144–151.
- Hosseini, P., Bahar, M., Madani, G. & Zirak, L. (2011). Molecular characterization of phytoplasmas associated with potato purple top disease in Iran. *J Phytopathol* **159**, 241–246.
- Iriti, M., Quaglino, F., Maffi, D., Casati, P., Bianco, P. A. & Faoro, F. (2008). *Solanum malacoxylon*, a new natural host of Stolbur phytoplasma. *J Phytopathol* **156**, 8–14.
- IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group (2004). 'Candidatus Phytoplasma', a taxon for the wall-less, non-helical prokaryotes that colonize plant phloem and insects. *Int J Syst Evol Microbiol* **54**, 1243–1255.
- Jones, P., Arocha, Y., Antesana, O., Montilliano, E. & Franco, P. (2005). First report of an isolate of 'Candidatus Phytoplasma australiense' associated with a yellow leaf roll disease of peach (*Prunus persicae*) in Bolivia. *Plant Pathol* **54**, 558.
- Jović, J., Cvrković, T., Mitrović, M., Krnjajić, S., Redinbaugh, M. G., Pratt, R. C., Gingery, R. E., Hogenhout, S. A. & Toševski, I. (2007). Roles of stolbur phytoplasma and *Reptalus panzeri* (Cixiinae, Auchenorrhyncha) in the epidemiology of Maize redness in Serbia. *Eur J Plant Pathol* **118**, 85–89.
- Kakizawa, S., Oshima, K., Nishigawa, H., Jung, H.-Y., Wei, W., Suzuki, S., Tanaka, M., Miyata, S., Ugaki, M. & Namba, S. (2004). Secretion of immunodominant membrane protein from onion yellows phytoplasma through the Sec protein-translocation system in *Escherichia coli*. *Microbiology* **150**, 135–142.
- Karimi, M., Contaldo, N., Mahmoudi, B., Duduk, B. & Bertaccini, A. (2009). Identification of stolbur-related phytoplasmas in grapevine showing decline symptoms in Iran. Le Progrès agricole et viticole, Hors Série, Extended abstract of the 16th Meeting ICVG, Dijon, France, 31 August–4 September, pp. 208–209.
- Kumar, S., Nei, M., Dudley, J. & Tamura, K. (2008). MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. *Brief Bioinform* **9**, 299–306.
- Langer, M. & Maixner, M. (2004). Molecular characterisation of Grapevine yellows associated phytoplasmas of the stolbur group based on RFLP-analysis of non-ribosomal DNA. *Vitis* **43**, 191–200.
- Lee, I.-M., Davis, R. E. & Gundersen-Rindal, D. E. (2000). Phytoplasma: phytopathogenic mollicutes. *Annu Rev Microbiol* **54**, 221–255.
- Lee, I.-M., Martini, M., Marcone, C. & Zhu, S. F. (2004). Classification of phytoplasma strains in the elm yellows group (16SrV) and proposal of 'Candidatus Phytoplasma ulmi' for the phytoplasma associated with elm yellows. *Int J Syst Evol Microbiol* **54**, 337–347.
- Lee, I.-M., Bottner, K. D., Secor, G. & Rivera-Varas, V. (2006). 'Candidatus Phytoplasma americanum', a phytoplasma associated with a potato purple top wilt disease complex. *Int J Syst Evol Microbiol* **56**, 1593–1597.
- Lee, I.-M., Bottner-Parker, K. D., Zhao, Y., Davis, R. E. & Harrison, N. A. (2010). Phylogenetic analysis and delineation of phytoplasmas based on *secY* gene sequences. *Int J Syst Evol Microbiol* **60**, 2887–2897.
- Liefting, L. W., Beever, R. E., Winks, C. J., Pearson, M. N. & Forster, R. L. S. (1997). Planthopper transmission of *Phormium* yellow leaf phytoplasma. *Australas Plant Pathol* **26**, 148–154.
- Liefting, L. W., Veerakone, S., Ward, L. I. & Clover, G. R. G. (2009). First report of 'Candidatus Phytoplasma australiense' in potato. *Plant Disease* **93**, 969.
- Maixner, M. (1994). Transmission of German grapevine yellows (Vergilbungskrankheit) by the planthopper *Hyalesthes obsoletus* (Auchenorrhyncha: Cixiidae). *Vitis* **33**, 103–104.
- Malembic-Maher, S., Salar, P., Filippin, L., Carle, P., Angelini, E. & Foissac, X. (2011). Genetic diversity of European phytoplasmas of the 16SrV taxonomic group and proposal of 'Candidatus Phytoplasma rubi'. *Int J Syst Evol Microbiol* **61**, 2129–2134.
- Marcone, C., Ragozzino, A. & Seemüller, E. (1997). Detection and identification of phytoplasmas infecting vegetable, ornamental and forage crops in southern Italy. *J Plant Pathol* **79**, 211–217.
- Martini, M., Lee, I.-M., Bottner, K. D., Zhao, Y., Botti, S., Bertaccini, A., Harrison, N. A., Carraro, L., Marcone, C. & other authors (2007). Ribosomal protein gene-based phylogeny for finer differentiation and classification of phytoplasmas. *Int J Syst Evol Microbiol* **57**, 2037–2051.
- Martini, M., Marcone, C., Mitrović, J., Maixner, M., Delić, D., Myrta, A., Ermacora, P., Bertaccini, A. & Duduk, B. (2012). 'Candidatus Phytoplasma convolvuli', a new phytoplasma taxon associated with bindweed yellows in four European countries. *Int J Syst Evol Microbiol* **62**, 2910–2915.
- Mertelik, J., Kloudova, K., Vanc, P., Mokra, V., Sediva, J., Navratil, M. & Valova, P. (2004). First detection of phytoplasmas in rhododendron in the Czech Republic. *Plant Disease* **88**, 906.
- Minucci, C. & Boccardo, G. (1997). Genetic diversity in the stolbur phytoplasma group. *Phytopathol Mediterr* **36**, 45–49.
- Oshima, K., Kakizawa, S., Nishigawa, H., Jung, H. Y., Wei, W., Suzuki, S., Arashida, R., Nakata, D., Miyata, S. & other authors (2004). Reductive evolution suggested from the complete genome sequence of a plant-pathogenic phytoplasma. *Nat Genet* **36**, 27–29.
- Padovan, A. C., Gibb, K. S., Bertaccini, A., Vibio, M., Bonfiglioli, R. E., Magarey, P. A. & Sears, B. B. (1995). Molecular detection of the Australian grapevine yellows phytoplasma and comparison with grapevine yellows phytoplasmas from Italy. *Aust J Grape Wine Res* **1**, 25–31.
- Padovan, A., Gibb, K. & Persley, D. (2000). Association of 'Candidatus Phytoplasma australiense' with green petal and lethal yellows diseases in strawberry. *Plant Pathol* **49**, 362–369.
- Quaglino, F., Zhao, Y., Bianco, P. A., Wei, W., Casati, P., Durante, G. & Davis, R. E. (2009). New 16Sr subgroups and distinct single nucleotide polymorphism lineages among grapevine Bois noir phytoplasma populations. *Ann Appl Biol* **154**, 279–289.
- Radonjić, S., Hrnčić, S., Jović, J., Cvrković, T., Krstić, O., Krnjajić, S. & Toševski, I. (2009). Occurrence and distribution of grapevine yellows caused by Stolbur phytoplasma in Montenegro. *J Phytopathol* **157**, 682–685.
- Riedle-Bauer, M., Sara, A. & Regner, F. (2008). Transmission of a Stolbur phytoplasma by the Agalliinae leafhopper *Anaceratagallia ribauti* (Hemiptera, Auchenorrhyncha, Cicadellidae). *J Phytopathol* **156**, 687–690.
- Sawayanagi, T., Horikoshi, N., Kanehira, T., Shinohara, M., Bertaccini, A., Cousin, M. T., Hiruki, C. & Namba, S. (1999). 'Candidatus phytoplasma japonicum', a new phytoplasma taxon associated with Japanese Hydrangea phyllody. *Int J Syst Bacteriol* **49**, 1275–1285.

- Seemüller, E. & Schneider, B. (2004).** 'Candidatus Phytoplasma mali', 'Candidatus Phytoplasma pyri' and 'Candidatus Phytoplasma prunorum', the causal agents of apple proliferation, pear decline and European stone fruit yellows, respectively. *Int J Syst Evol Microbiol* **54**, 1217–1226.
- Seemüller, E., Schneider, B., Mäurer, R., Ahrens, U., Daire, X., Kison, H., Lorenz, K. H., Firrao, G., Avinent, L. & other authors (1994).** Phylogenetic classification of phytopathogenic mollicutes by sequence analysis of 16S ribosomal DNA. *Int J Syst Bacteriol* **44**, 440–446.
- Šeruga, M., Ćurković Perica, M., Škorić, D., Kozina, B., Mirošević, N., Šarić, A., Bertaccini, A. & Krajačić, M. (2000).** Geographic distribution of Bois Noir phytoplasmas infecting grapevines in Croatia. *J Phytopathol* **148**, 239–242.
- Streten, C. & Gibb, K. S. (2005).** Genetic variation in *Candidatus Phytoplasma australiense*. *Plant Pathol* **54**, 8–14.
- Suzuki, S., Oshima, K., Kakizawa, S., Arashida, R., Jung, H.-Y., Yamaji, Y., Nishigawa, H., Ugaki, M. & Namba, S. (2006).** Interaction between the membrane protein of a pathogen and insect microfilament complex determines insect-vector specificity. *Proc Natl Acad Sci U S A* **103**, 4252–4257.
- Terlizzi, F., Babini, A. R. & Credi, R. (2006).** First report of stolbur phytoplasma (16SrXII-A) on strawberry in northern Italy. *Plant Disease* **90**, 831.
- Torres, E., Botti, E., Rahola, J., Martin, M. P. & Bertaccini, A. (2005).** Grapevine yellows diseases in Spain: eight year survey of disease spread and molecular characterization of phytoplasmas involved. *An Jard Bot Madr* **62**, 127–133.
- Valiunas, D., Staniulis, J. & Davis, R. E. (2006).** 'Candidatus Phytoplasma fragariae', a novel phytoplasma taxon discovered in yellows diseased strawberry, *Fragaria x ananassa*. *Int J Syst Evol Microbiol* **56**, 277–281.
- Weisburg, W. G., Tully, J. G., Rose, D. L., Petzel, J. P., Oyaizu, H., Yang, D., Mandelco, L., Sechrest, J., Lawrence, T. G. & other authors (1989).** A phylogenetic analysis of the mycoplasmas: basis for their classification. *J Bacteriol* **171**, 6455–6467.
- White, D. T., Blackall, L. L., Scott, P. T. & Walsh, K. B. (1998).** Phylogenetic positions of phytoplasmas associated with dieback, yellow crinkle and mosaic diseases of papaya, and their proposed inclusion in 'Candidatus Phytoplasma australiense' and a new taxon, 'Candidatus Phytoplasma australasia'. *Int J Syst Bacteriol* **48**, 941–951.
- Zirak, L., Bahar, M. & Ahoonmanesh, A. (2009).** Molecular characterization of phytoplasmas related to peanut witches broom and stolbur groups infecting plum in Iran. *J Plant Pathol* **91**, 713–716.
- Zirak, L., Bahar, M. & Ahoonmanesh, A. (2010).** Molecular characterization of phytoplasmas associated with peach diseases in Iran. *J Phytopathol* **158**, 105–110.
- Zwolinska, A., Krawczyk, K. & Pospieszny, H. (2012).** Molecular characterization of stolbur phytoplasma associated with pea plants in Poland. *J Phytopathol* **160**, 317–323.