Phytopathology

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Journal:	*Phytopathology*
Manuscript ID	PHYTO-06-17-0215-R.R2
Manuscript Type:	Research
Date Submitted by the Author:	06-Oct-2017
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Keywords:	Ecology and epidemiology, Population biology

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Molecular typing of 'bois noir' phytoplasma strains in the *Chianti Classico* area (Tuscany, central Italy) and their association with symptom severity in *Vitis vinifera* L. cv. Sangiovese

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ABSTRACT

Bois Noir (BN) is the most widespread disease of the grapevine yellows complex in the Euro-Mediterranean area. BN is caused by '*Candidatus* Phytoplasma solani' (BNp), transmitted from herbaceous plants to grapevine by polyphagous insect vectors. In this study, genetic diversity among BNp strains, their prevalence and possible association with grapevine symptom severity were investigated in a Sangiovese clone organic vineyard, in the *Chianti Classico* area (Tuscany). Field surveys over two years revealed a range of symptom severity on grapevine and an increase of BN incidence. TaqMan allelic discrimination assay detected only *tufB*-type b among BNp strains, suggesting the prevalence of the bindweed-related ecology. Nucleotide sequence analyses of *vmp1* and *stamp* genes identified 12 *vmp1* and 16 *stamp* sequence variants, showing an overall positive selection for such genes. The prevalent genotype was Vm43/St10, reported for the first time in this study and closely related to strains identified only in the French Eastern Pyrenees. BNp strains, identified in the examined vineyard and mostly grouped in separate bindweed-related phylogenetic clusters, showed statistically significant differences in their distribution in grapevines exhibiting distinct symptom severity. These results suggest the possible occurrence of a range of virulence within BNp strain populations in the *Chianti Classico* area.

Additional Keywords: grapevine yellows; '*Candidatus* Phytoplasma solani'; sequence variants; membrane protein; multiple gene typing

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INTRODUCTION

Grapevine yellows (GY) are phytoplasma-associated diseases occurring worldwide in the main grapevine-growing areas. Among GY, Bois Noir (BN) is one of the most important diseases, responsible for serious crop losses in the Euro-Mediterranean area and in several other countries (Belli et al. 2010). The causal agent of BN was identified in phytoplasma (BNp) strains belonging to the species '*Candidatus* Phytoplasma solani' ('*Ca.* P. solani'), 16SrXII-A subgroup (Quaglino et al. 2013). Disease symptoms consist of plant decline, shrivelled grapes, leaf rolling, desiccation of inflorescences, irregular ripening of wood, and reddening or yellowing of leaves on red and white cultivars, respectively (Belli et al. 2010). BN is mainly transmitted to grapevine (*Vitis vinifera* L.) by the cixiid planthopper *Hyalesthes obsoletus* Signoret (Sharon et al. 2005) which has a biological cycle that is completed preferentially on field bindweed (*Convolvulus arvensis* L.) and common nettle (*Urtica dioica* L.). In fact, infected grapevine is considered a "dead-end host" of BNp since its main vector feeds on this species only occasionally, reducing its role in the disease epidemiology. BN spreads also in areas where *H. obsoletus*, the main vector, does not occur (Belli et al. 2010; Maixner et al. 2011), and recent studies have proven that *Reptalus panzeri* Löw and *R. quinquecostatus* Dufour can act as vectors of BNp to grapevine in Serbian and French vineyards, respectively (Cvrkovic et al. 2014; Chuche et al. 2016). Moreover, other studies have also identified several herbaceous plant hosts playing a direct role in BN diffusion (Berger et al. 2009; Marchi et al. 2015; Mori et al. 2015).

Such evidence, reporting the presence of multiple BNp plant and insect hosts in the Euro-Mediterranean area, indicate that this phytoplasma exists in varied ecosystems, where selection conceivably alters strain populations (Quaglino et al. 2013, 2017). This hypothesis implies that ecological relationships of BNp and BNp-related strains can be reflected in intra-species strain diversity. Thus, in the last years, numerous studies focused on distinguishing genetic structure of BNp strains with the aim to identify strain-specific molecular markers associable to distinct biological features. Such studies were carried out by nucleotide sequence analyses of the genes *tufB*, encoding the translation elongation factor Tu (Schneider et al. 1997), *secY*, encoding a translocation protein (Fialovà et al. 2009), *vmp1* and *stamp*, encoding membrane proteins presumably involved in the interaction between BNp and its host(s) (Cimerman et al. 2009; Fabre et al. 2011).

Information obtained from the *tufB* gene characterization allowed the identification of two main BNp *tufB*-types associated with herbaceous plant hosts related to distinct epidemiological systems in Europe (Langer and Maixner 2004; Belli et al. 2010; Maixner 2011): (i) *tufB*-type a, associated to *U. dioica* and prevalent in western Europe and northern Italy (Quaglino et al. 2013), (ii) *tufB-type* b,

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mainly associated to *C. arvensis* and many other herbaceous hosts and prevalent in central-southern Italy (Pacifico et al. 2009; Murolo et al. 2016). Furthermore, the analyses of nucleotide sequences of the genes *secY*, *stamp*, and *vmp1* evidenced a larger variability among BNp strains within the two main *tufB*-types (Foissac et al. 2013; Kostadinovska et al. 2014; Murolo and Romanazzi 2015). Currently, 23 V-types distinct by *vmp1* restriction fragment length polymorphism (RFLP) profiles (nomenclature determined according to SEE-ERANET, X. Foissac, INRA, Bordeaux, France), 63 nucleotide sequence variants for *vmp1* gene and 35 for *stamp* gene were described among BNp and '*Ca*. P. solani' strains (Murolo and Romanazzi 2015; Quaglino et al. 2016). Utilization of molecular markers identified in these studies increased the knowledge of BNp strains movements and host range (Crvković et al. 2014; Kosovać et al. 2015), and confirmed the presence of a positive selective pressure determining the BNp strain population complexity in different vineyard agro-ecosystems (Murolo et al. 2014). Moreover, recent evidence reinforced the hypothesis that BNp strains, distinguishable using such molecular markers, can exhibit a range of virulence associated with different symptom severity in infected grapevine plants (Quaglino et al. 2016).

The main objectives of this study, carried out over two following seasons in a Sangiovese clone organic vineyard in the *Chianti Classico* area (Greve in Chianti, Tuscany - central Italy), were to investigate the genetic diversity among BNp strains through multiple gene typing and to evaluate their possible association with grapevine symptom severity by statistical analyses.

MATERIALS AND METHODS

Vineyard statement

The study was conducted within an experimental area (735 plants), selected as representative of a vineyard planted in 1997 in Greve in Chianti (FI), area localized in the traditional grapevine-growing region of *Chianti Classico*, Tuscany (central Italy) (43° 33’ 21’’ N, 11° 18’ 8’’ E; 460 m a.s.l.). The vineyard was bordered by other vineyards and forests and was conducted according to organic management. Vines (*Vitis vinifera* L. cv. Sangiovese, I-SS F9 A5 48) were trained as cordon (planting density 2.3 m between the rows, 0.8 m along the row).

Symptom observations and plant samples

In 2015 and 2016, each vine was visually assessed for the presence of GY disease once in June and once in September of each year. The severity of symptoms was classified and sorted according

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to the GY symptomatic scale from zero to three modified from the one proposed by Riedle-Bauer and colleagues (2010) as follows: (i) symptom severity class 0: healthy plants with no symptoms; (ii) symptom severity class 1: one shoot with mild leaf symptoms; (iii) symptom severity class 2: 2-3 shoots with leaf symptoms; (iv) symptom severity class 3: more than 3 shoots with leaf symptoms and berry shrivel. Furthermore, overall disease severity in the vineyard was evaluated using the formula $S = \Sigma (c \cdot f) / n$, proposed by Murolo and Romanazzi in 2015, where: S is disease severity, c is symptom severity class, f is the frequency of the symptom severity class, and n is the number of symptomatic plants. For each year, ten leaves were sampled from each symptomatic plants and from 10 randomly selected symptomless plants for phytoplasma detection and characterization.

V. vinifera cv. Sangiovese, obtained from the screenhouse of the Department of Agriculture, Food and Environment (DAFE, University of Pisa, Italy), was used as healthy control plants; *V. vinifera* plants, previously found infected by either '*Ca*. P. solani' (subgroup 16SrXII-A) or Flavescence dorée phytoplasmas (FDp) (subgroups 16SrV-C or -D), were used as infected reference control (IC).

Fresh central midribs of fully developed leaves were dissected and stored at -20°C until total nucleic acids (TNA) extraction.

DNA extraction

DNA was extracted with cetyltrimethylammonium bromide (CTAB) based buffer from leaf veins according to the protocol described by Li et al. (2008), with some modifications. Briefly, leaf veins (1 g) were homogenized in plastic bags (Bioreba, Switzerland) with 7 ml of 2% CTAB buffer using Homex 6 (Bioreba, Switzerland). The homogenate was incubated at 65°C for 15 minutes. DNA was extracted by one volume of chloroform:iso-amylalcohol (24:1) and precipitated with one volume of isopropanol. Pellets were washed with 70% ethanol, air-dried, suspended in 100 µl of deionized water and stored at -20°C until use.

GY phytoplasmas detection and relative quantification

Specific detection of phytoplasmas associated with BN and FD, the GY diseases more commonly present in Italy, was carried out by amplification of 16S ribosomal DNA through TaqMan assay using the G8830A AriaMx Real-time PCR (Agilent Technologies, USA) following reaction conditions as described by Angelini et al. (2007). The template used in the assay was a 1:10 dilution of the DNA extracted from the samples. The grapevine chloroplast chaperonin 21

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gene and total nucleic acids extracted from healthy control plants and IC were used as endogenous, negative and positive controls, respectively. Threshold cycle (Ct) < 37 was associated with the presence of GY phytoplasmas (Mori et al. 2015). As in previous studies (Baric 2012; Minguzzi et al. 2016), the relative quantification of phytoplasmas in each sample was calculated using the following formula: $\Delta Ct = Ct_p - Ct_g$, where ΔCt is the normalized value, Ct_p is the Ct obtained from amplification of phytoplasmatic *16S rRNA* gene, and Ct_g is the Ct obtained from amplification of grapevine chaperonin gene, the endogenous control used in the reaction. ΔCt values were compared through one-way ANOVA, followed by Tukey's Exact Test, performed in SPSS statistical package for Windows, v. 24.0 (IBM Corporation, Armonk, NY) to determine if the symptom severity of the disease was correlated to different phytoplasma relative abundance in the plants. Correlation coefficient (R^2) was calculated by linear regression model.

BNp characterization by multiple gene sequence analyses

'*Ca*. P. solani' [Bois noir phytoplasma (BNp)] strains, detected in grapevine plants, were characterized by nucleotide sequence analyses of the genes *tufB*, *vmp1* and *stamp*.

Identification of *tufB*-types, commonly present in Italy (*tufB*-a and *tufB*-b) (Mori et al. 2015), was performed using the TaqMan allelic discrimination assay, employing *tufB*-type specific probes carrying different fluorescent dyes, according to Berger and colleagues (2009).

The *vmp1* gene was amplified in an automated thermal cycler C1000 Cycler Touch (Biorad, Italy) using the StolH10F1/StolH10R1 primer pair (Cimerman et al. 2009) followed by nested PCR with the TYPH10F/TYPH10R primer pair, using mixtures and PCR conditions as described by Fialovà et al. (2009). Nested PCR amplicons were verified through electrophoresis on 1% agarose gels in Tris-borate-EDTA (TBE) buffer. Then, restriction fragment length polymorphism (RFLP) analysis was performed using the *Rsa*I restriction enzyme (Pacifico et al. 2009), according to the manufacturer's instructions (New England BioLabs, USA). Digestion fragments were separated through electrophoresis on 3% agarose gels in TBE buffer stained with Gel-Red (Biotum, USA) and visualized under UV transilluminator. Attribution of BNp strains, identified in the present study, to *vmp1* RFLP types (V-types) was determined by comparison of their *Rsa*I-RFLP patterns with *vmp1* digestion profiles previously described in accordance with SEE-ERANET nomenclature (Foissac et al. 2013; Quaglino et al. 2016). *vmp1* amplicons, representative of the identified V-types, were sequenced (5X coverage per base position) by a commercial service (Eurofins Genomics, Germany). Nucleotide sequences were assembled by the Contig Assembling Program and trimmed to the annealing sites of the primers TYPH10F/TYPH10R in the software BioEdit, version 7.2.6

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(Hall 1999). To confirm the attribution to V-types, trimmed nucleotide sequences were searched for single-nucleotide polymorphisms in recognition sites of the enzyme *Rsa*I through virtual RFLP analyses using the software pDRAW32 (http://www.acaclone.com/). Moreover, *vmp1* nucleotide sequences were aligned using ClustalW Multiple Alignment and analysed by Sequence Identity Matrix in the software BioEdit. Attribution to *vmp1* sequence variants was carried out by comparison with sequences previously deposited in GenBank database. In detail, nucleotide sequences of the same variant share 100% sequence identity.

The *stamp* gene was amplified in an automated thermal cycler C1000 Cycler Touch using StampF/StampR0 and StampF1/ StampR1 primer pairs in direct and nested PCR, respectively, following PCR reaction conditions as described by Fabre et al. (2011). Nested PCR amplicons were verified through electrophoresis on 1% agarose gels in TBE buffer. All obtained *stamp* amplicons were sequenced and analysed as described for *vmp1* gene.

To further characterize the *vmp1* and *stamp* gene sequence variants, their nucleotide sequences were translated *in silico* and searched for the presence of non-synonymous and synonymous single nucleotide polymorphisms (SNPs), and other sequence modifications (insertions/deletions).

Collective *vmp1*/*stamp* types were determined combining the *vmp1* and *stamp* sequence variant of each BNp strain identified in grapevine, as previously described (Quaglino et al. 2016).

Phylogenetic analysis, association of BNp strains with symptom severity, and selective pressure on BNp strain population

Representative nucleotide sequences of *vmp1* and *stamp* sequence variants, identified in this and previous studies (Quaglino et al. 2016), were utilized for phylogenetic analyses. Moreover, representative nucleotide sequences of *vmp1* and *stamp* genes were concatenated by BioEdit and employed for phylogenetic analyses. In detail, unrooted phylogenetic trees were generated by minimum evolution method carried out using the Jukes-Cantor model and bootstrap replicated 1000 times in the MEGA7 software (Tamura et al. 2013).

The association between BNp strains (identified in 2016 and grouped in distinct *vmp1*, *stamp*, and *vmp1*/*stamp* phylogenetic clusters) and BN symptom severity was determined as the difference in their distribution in grapevines showing symptom severity class 1, 2, and 3 in 2016 through statistical analyses using a χ^2 test (p<0.10) in SPSS.

Moreover, distinct *vmp1*, *stamp* and *vmp1*/*stamp* phylogenetic clusters, including BNp strains identified in 2016, were ranked in accordance with (i) their average disease severity (A*S*), calculated using the formula $AS = \Sigma\ (c \cdot f^{AS}) / n^{AS}$, where: A*S* is the average disease severity of the

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cluster, *c* is symptom severity class, f^{AS} is the frequency of the symptom severity class in grapevines infected by BNp strains of the cluster, and n^{AS} is the number of symptomatic grapevines infected by BNp strains of the cluster, and (ii) their overall disease severity percentage (O*S*%), calculated using the formula $OS\% = [\Sigma\ (c \cdot f^{AS}) / n] \cdot 100 / S$, where: O*S*% describes how much each cluster contributes to the disease severity in the vineyard, *c* is symptom severity class, f^{AS} is the frequency of the symptom severity class in grapevines infected by BNp strains of the cluster, *n* is the total number of symptomatic grapevines, and *S* is disease severity (as described above).

Codon based Z-test of positive selection was performed using the Nei-Gojobori method with MEGA7 to determine *dN/dS* ratio and to calculate the probability of rejecting the null hypothesis of strict-neutrality (*dN* = *dS*) in favor of the positive selection hypothesis (*dN* > *dS*). *dS* and *dN* are the numbers of synonymous and nonsynonymous substitutions per site, respectively. The variance of the difference was computed using the bootstrap method (1000 replicates). Analyses were conducted according to Nielsen (2005), Murolo and Romanazzi (2016). The overall *dN/dS* ratio > 1.0 and p value < 0.05 means positive selection, while ratio = 1 or < 1.0 means neutral or purifying selection process, respectively (Nei and Kumar, 2000; Murolo et al. 2014). The analysis involved 40 and 66 nucleotide sequences of the genes *vmp1* and *stamp*, respectively. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 517 and 158 codon positions in the final dataset for the genes *vmp1* and *stamp*, respectively.

RESULTS

Symptom observations

In 2015, 24 out of 735 (3.3%) grapevine plants showed typical GY symptoms. Symptom severity classes were observed in the vineyard and the most represented was the symptom severity class 2 (37.5% of the symptomatic vines) followed by symptom severity class 3 (33.3%) and y class 1 (29.2%) (Table 1).

In 2016, 53 out of 734 (7.2%) grapevine plants showed typical GY symptoms: (i) 33 plants (out of 53) exhibited GY symptoms for the first time; (ii) 20 plants (out of 53) showed symptoms in both years; (iii) three plants, showing symptoms in 2015, were symptomless in 2016; (iv) one plant, showing symptoms in 2015, was eradicated after death. As in 2015, all the three symptom severity classes were observed in the vineyard and the most represented was the symptom severity class 3

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(47.2% of the symptomatic vines) followed by symptom severity class 1 and 2 which were equally abundant (26.4% each). Overall disease severity (*S*) was determined as 2.04 in 2015 and 2.20 in 2016.

GY phytoplasma detection and relative quantification

Real-time PCR, carried out by TaqMan assays performed using primer pairs specific for the amplification of BNp and FDp 16S rDNA, detected BNp in every symptomatic grapevine plant in 2015, while in 2016 it was detected in 45 out of 53 symptomatic grapevine plants. In both years, FDp was never detected in symptomatic plants. BNp and FDp were never detected in symptomless plants, including the three plants that no longer showed symptoms in 2016. In both years, Ct values obtained by amplification of BNp 16S rDNA varied among samples ($27 < Ct < 34$), while those obtained for chaperonin were slightly less variable ($16 < Ct < 20$); ΔCt values were from 2 to 11 (Table 1, 2). Assay reliability was confirmed by the results obtained from the controls: (i) healthy control plants and reaction mixtures devoid of total nucleic acids gave no amplification; (ii) IC gave expected amplification for BNp (average Ct 31) and FDp subgroup 16SrV-C (average Ct 28) and -D (average Ct 28). No statistically significant differences ($R^2 = 0.008$; $p = 0.57$) between symptom severity of grapevine and ΔCt values were obtained.

BNp strain characterization by multiple gene typing

tufB gene molecular characterization was carried out on BNp-infected grapevine plants (24 in 2015 and 45 in 2016). TaqMan allelic discrimination assays revealed that BNp strains, infecting symptomatic grapevines in both years, are classified as *tufB*-type b.

Out of 69 BNp strains, identified in 24 and 45 symptomatic vines respectively in 2015 and in 2016, 57 (15 out of 24 from 2015, and 42 out of 45 from 2016) yielded *vmp1* nested-PCR amplicons (TYPH10F/TYPH10R) that were typed through digestion using the *Rsa*I enzyme. BNp strains identified in 2015 showed the presence of two *Rsa*I-RFLP profiles attributed to *vmp1* types V11 (6 strains) and V12 (9 strains). BNp strains identified in 2016 showed the presence of three actual *Rsa*I-RFLP profiles attributed to *vmp1* types V11 (24 strains), V12 (15 strains), and V9 (3 strains). Comprehensive attribution to V-type was confirmed by *in silico* RFLP analysis (Figure 1). Ten BNp strains, identified in the same symptomatic plants in both years, showed undistinguishable *Rsa*I-RFLP patterns.

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vmp1 sequence analysis was carried out on 40 BNp strains (9 from 2015, 31 from 2016), representative of the V-types identified by RFLP digestions (Table 1, 2). Based on sequence identities, 12 *vmp1* sequence variants (here named vmFi1 to vmFi12) have been identified within BNp strain populations. Sequence variants vmFi1 (prevalent in both years), vmFi2, and vmFi3 were present in both years; vmFi4 was detected only in 2015, while vmFi5 to vm12 only in 2016 (Table 1, 2). Comparison with *vmp1* sequence variant dataset, updated in the present study (Supplementary Table S1), revealed that vmFi1, vmFi2, vmFi3, vmFi5, and vmFi6 shared 100% sequence identity with previously reported sequence variants Vm43, Vm45, Vm42, Vm39, and Vm41, respectively. Other *vmp1* sequence variants (vmFi4, vmFi7 to vmFi12), reported in the present study, were not identical to any other *vmp1* sequences in the dataset but shared the best identities versus sequence variants Vm39, Vm41, Vm42, Vm43 and Vm45. Further analyses, carried out on nucleotide and *in silico* translated *vmp1* gene sequences, evidenced that the genetic diversity among sequence variants vmFi1 to vmFi12 is caused by the presence of 125 SNPs (116 non-synonymous, 9 synonymous) and 5 insertions determining different lengths of the gene *vmp1* and the coded Vmp1 protein (Supplementary Figure S1 and Supplementary Table S2). New *vmp1* sequence variants shared high nucleotide sequence identity (99.4 to 99.9%) but were distinguished from closely related sequence variants previously published (Vm39, Vm41, Vm42, Vm43 and Vm45) by non-synonymous SNPs (Supplementary Table S3). For each *vmp1* sequence variant, one representative nucleotide sequence was deposited to NCBI GenBank at Accession Number shown in Table 3 (named from vmFi1 to vmFi12).

Out of 69 BNp strains, identified in 24 and 45 symptomatic vines respectively in 2015 and in 2016, 66 (22 from 2015, and 44 from 2016) yielded *stamp* nested-PCR amplicons (StampF1/StampR1) of the expected size (about 500 bp) that were typed by nucleotide sequence analysis. Based on sequence identities, 16 *stamp* sequence variants (here named stFi1 to stFi16) have been identified within BNp strain populations. Sequence variants stFi1 (prevalent in 2016), stFi2 (prevalent in 2015), stFi3, and stFi4 were present in both years; stFi5 to stFi9 were detected only in 2015, while stFi10 to stFi16 only in 2016 (Table 1, 2). Comparison with *stamp* sequence variant dataset, updated in the present study (Supplementary Table S4), revealed that stFi1, stFi2, stFi3, and stFi4 shared 100% sequence identity with previously reported sequence variants St10, St5, St18, and St22, respectively. Other *stamp* sequence variants (stFi5 to stFi16), reported in the present study, are not identical to any other *stamp* sequences in the dataset but shared the best identities versus sequence variants St5, St10, St15, St18, St22, St36 and St37. Further analyses, carried out on nucleotide and *in silico* translated *stamp* gene sequences, evidenced that the genetic diversity among sequence variants stFi1 to stFi16 is caused by the presence of 50 SNPs (42 non-

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synonymous, 8 synonymous) and 1 insertion (nt 253-256) (Supplementary Figure S2). New *stamp* sequence variants shared high nucleotide sequence identity (95.9 to 99.7%) but were distinguished from closely related sequence variants previously published (St5, St10, St15, St18, St22, St36 and St37) by non-synonymous SNPs (Supplementary Table S5). For each *stamp* sequence variant, one representative nucleotide sequence was deposited to NCBI GenBank at Accession Number shown in Table 4 (named from stFi1 to stFi16).

Through the combination of *vmp1* and *stamp* nucleotide sequences, available for 40 BNp strains over the two years, 17 *vmp1/stamp* types were identified. The type vmFi1/stFi1 (identical to Vm43/St10) represents the most widespread type combination for both years: 55.5% in 2015 and 35.3% in 2016.

Phylogenetic analysis, association of BNp strains with symptom severity, and selective pressure on BNp strain population

A phylogenetic tree, generated employing representative *vmp1* sequence variants from dataset (Vm1 to Vm80, Supplementary Table S1) and from the present study (vmFi1 to vmFi12), identified the presence of four main clusters. Sequence variants, here identified in symptomatic grapevines, grouped into clusters 1 (vmFi2, vmFi3, vmFi4, vmFi6, vmFi7, vmFi8, vmFi12), 2 (vmFi5, vmFi9), and 3 (vmFi1, vmFi10, vmFi11) (Fig. 2A). Statistically significant differences were observed in the distribution of BNp strains, belonging to *vmp1* phylogenetic cluster 3 (A*S* 2.37, O*S*% 32.5), in grapevine plants showing symptom severity class 1, 2 and 3 ($\chi^2 = 4.667$, $p = 0.097$). No statistically significant differences were observed in the distribution of BNp strains, belonging to *vmp1* phylogenetic clusters 1 (A*S* 2.42, O*S*% 24.8), and 2 (A*S* 2.67, O*S*% 6.8) (Fig. 2B).

A phylogenetic tree, generated employing representative *stamp* sequence variants from dataset (St1 to St46, Supplementary Table S4) and from the present study (stFi1 to stFi16), identified the presence of three main clusters. Sequence variants, here identified in symptomatic grapevines, grouped into clusters 1 (stFi3, stFi6, stFi8, stFi10, stFi16), 2 (stFi2, stFi4, stFi11, stFi15), 3 (stFi1, stFi5, stFi9, stFi12, stFi13, stFi14); sequence variant stFi7 did not group in identified clusters (Fig. 3A). Statistically significant differences were observed in the distribution of BNp strains, belonging to *stamp* phylogenetic cluster 1 (A*S* 2.60, O*S*% 22.2), in grapevine plants showing symptom severity class 1, 2 and 3 ($\chi^2 = 8$, $p = 0.018$). No statistically significant differences were observed in the distribution of BNp strains, belonging to *stamp* phylogenetic clusters 2 (A*S* 2.47, O*S*% 31.6) and 3 (A*S* 2.21, O*S*% 35.9) (Fig. 3B).

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A phylogenetic tree, generated employing concatenated *vmp1* and *stamp* gene nucleotide sequences representative of 66 *vmp1/stamp* types [49 from dataset (Supplementary Table S6) and 17 from the present study], identified the presence of eight main clusters. *vmp1/stamp* types, here identified in symptomatic grapevines, grouped into clusters 1 (vmFi1/stFi1, vmFi1/stFi12, vmFi1/stFi13, vmFi1/stFi14, vmFi10/stFi1, vmFi11/stFi1), 3 (vmFi2/stFi3, vmFi8/stFi3, vmFi12/stFi2, vmFi12/stFi3), 4 (vmFi3/stFi2, vmFi3/stFi4, vmFi4/stFi4, vmFi6/stFi2, vmFi7/stFi3), and 5 (vmFi5/stFi2, vmFi9/stFi2) (Fig. 4A). Further analyses of the nucleotide sequence alignment of *vmp1/stamp* types, identified in the *Chianti Classico* area, revealed that BNp strains within each clusters (intra-cluster heterogeneity) shared a mean sequence identity of 99.57% (about 10 SNPs distinguishing one strain to another), while BNp strains of distinct clusters (inter-cluster heterogeneity) shared a mean sequence identity of 89.41% (about 184 SNPs distinguishing one strain to another) (Table 5). Statistically significant differences were observed in the distribution of BNp strains, belonging to *vmp1*/*stamp* phylogenetic cluster 1 [(χ^2 = 4.667, p = 0.097), (A*S* 2.37, O*S*% 32.5)] and 3 [(χ^2 = 7, p = 0.030), (A*S* 2.67, O*S*% 13.7)] in grapevine plants showing symptom severity class 1, 2 and 3. Instead, no statistically significant differences were observed in the distribution of BNp strains belonging to *vmp1*/*stamp* phylogenetic clusters 4 (A*S* 2.16, O*S*% 11.1), and 5 (A*S* 2.67, O*S*% 6.8) (Fig. 4B).

The overall ratio between the non-synonymous to the synonymous mutations (*dN/dS*) was >1 for both genes analysed. In detail, for *vmp1* gene *dN/dS* test showed ratio = 2.482, *P* = 0.014 and for *stamp* gene *dN/dS* = 2.368, *P* = 0.019. These results confirmed the high number of non-silent (*dN*) mutations revealed by sequence analyses.

DISCUSSION

Real-time PCR-based detection of GY phytoplasmas revealed that symptomatic grapevines, observed in the vineyard examined in the present study, were infected by BNp, excluding the presence of phytoplasmas associated with Flavescence dorée, previously reported in Tuscany (Belli et al. 2010). On the other hand, negative PCR results from symptomatic grapevines could be connected with the low abundance and/or sporadic distribution of phytoplasmas in symptomatic plant tissues (Constable et al. 2003).

Recent studies highlighted that the impact of BN on the vineyards and on the distribution of symptomatic grapevines is influenced by two main driving forces: the transmission of BNp from infected source plant(s) to grapevines and the spontaneous remission of symptoms (possibly driving to recovery) of diseased grapevines (Murolo et al. 2014; Mori et al. 2015). Field symptom

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observations evidenced that BN impact on the examined Sangiovese vineyard, localized in the *Chianti Classico* area of Tuscany, doubled from 2015 to 2016 (from 3.3% to 7.2% of symptomatic grapevines), indicating the high frequency of BNp transmission to grapevines as the main epidemiological driving force acting on BN incidence.

Molecular typing, carried out by analysing *tufB*, *vmp1* and *stamp* gene nucleotide sequences, revealed a great intra-species strain diversity among BNp strains, identified in symptomatic grapevines, the complexity of which increased over the two years. In detail, TaqMan real-time PCR assays, differentiating the two main BNp *tufB*-types present in Italy, underlined the unique presence of BNp *tufB*-type b in the examined vineyard. This evidence is in accordance with data reporting the prevalence of *tufB*-type b in vineyards of Central and Southern Italy (Pacifico et al. 2007; Marchi et al. 2015; Murolo and Romanazzi 2015). Due to the main association of *tufB*-types (a and b) with distinct epidemiological systems (Langer and Maixner 2004), it is reasonable to hypothesize that BNp ecology, in the Sangiovese vineyard under study, can be prevalently related to the host system *Convolvulus arvensis* - *Hyalesthes obsoletus* - *Vitis vinifera*.

A deeper understanding of the degree of genetic variability among the BNp strains was obtained by the characterization of the *vmp1* and *stamp* gene sequences. Based on *Rsa*I-RFLP profiles of the *vmp1* gene amplicons, 3 main V-types were identified as widespread in both years (V11, V12) or sporadic only in 2016 (V9). Previous studies reported the large presence of the type V12 in Tuscany, Piedmont and Marche, V11 in Sardinia, and V9 in Sicily and Piedmont (Pacifico et al. 2009).

Based on sequence identity of *vmp1* and *stamp* gene sequences retrieved from NCBI GenBank, it is possible to determine the presence of 80 *vmp1* and 46 *stamp* genetic variants among '*Ca*. P. solani' strains (Quaglino et al. 2016; this study). Unexpectedly, in the examined Sangiovese vineyard in Tuscany, BNp strain populations showed more variability in *stamp* gene (16 sequence variants, of which 12 have been reported for the first time) compared to *vmp1* gene (12 sequence variants, of which seven reported for the first time in this study) nucleotide sequences.

In BNp strain populations, identified in both years, the prevalent *vmp1* sequence variant (identified in 50% of the BNp strains) was vmFi1, identical to the sequence variant Vm43 (Quaglino et al. 2016), previously reported only in '*Ca*. P. solani' strains infecting *Linaria vulgaris* and *H. obsoletus* in Italy (Marchi et al. 2015; Landi et al. 2015). Phylogenetic analyses revealed that BNp strains of vmFi1 to vmFi12 sequence variants, identified in Tuscany in this study, grouped in clusters *vmp1*-1, -2, and -3 along with previously reported sequence variants including BNp and '*Ca*. P. solani' strains associated with bindweed-related host systems in Europe (Cimerman et al. 2009; Pacifico et al. 2009; Murolo and Romanazzi 2015; Quaglino et al. 2016).

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In BNp strain populations, identified in both years, the *stamp* sequence variants stFi1, stFi2 and stFi3 (identical to sequence variants St10, St5 and St18, respectively) were largely prevalent (identified in 85% of the BNp strains) (Table 4). Sequence variant St5 was already reported in central-eastern Italy (along with St18) by Murolo and Romanazzi (2015) and widely spread in Slovenia (Fabre et al. 2011), Germany (Fabre et al. 2011; Johannesen et al. 2012), Austria (Aryan et al. 2014) and Macedonia (Atanasova et al. 2015). Intriguingly, sequence variant St10, the occurrence of which in symptomatic plants increased the most over the two years, was never reported in Italy before this study. In fact, previous studies reported St10 only on *Solanum lycopersicum* and *H. obsoletus* in the French Eastern Pyrenees (Fabre et al. 2011). Phylogenetic analyses revealed that BNp strains of stFi1 to stFi16 sequence variants, identified in Tuscany in this study, grouped in all three *stamp* clusters with previously reported sequence variants including BNp and '*Ca*. P. solani' strains mainly associated with bindweed-related host systems in Europe (Fabre et al. 2011; Cvrković et al. 2014; Kosovac et al. 2015; Murolo and Romanazzi 2015; Quaglino et al. 2016).

As reported in previous studies (Durante et al. 2012; Quaglino et al. 2016), *vmp1* and *stamp* gene concatenated nucleotide sequences have been employed to improve the robustness of phylogenetic analyses. Within BNp strains infecting examined grapevines, combination of *vmp1* and *stamp* sequence variants allowed the identification of 17 *vmp1*/*stamp* types grouped in clusters *vmp1*/*stamp*-1, -3, -4, -5. In detail, the prevalent type vmFi1/stFi1, constituted by the unreported combination of two known sequence variants present in Italy (Vm43) and in French Eastern Pyrenees (St10), grouped in cluster *vmp1*/*stamp*-1 with *vmp1*/*stamp* types identified in *H. obsoletus*, grapevine and other host plants outside of Italy. The overall ratio between the non-synonymous to the synonymous mutations showed that *vmp1* and *stamp* genes in '*Ca.* P. solani' strains in Tuscany are under positive selection process. In contrast with previous studies reporting differences in intensive selection acting on *vmp1* and *stamp* genes (Murolo and Romanazzi 2015; Quaglino et al. 2016), the values of *dN*/*dS* ratio indicated a similar intensity of selection for both genes in BNp strain populations from Tuscany.

Two main hypotheses can be formulated to explain the surprising prevalent spread of the BNp *vmp1*/*stamp* type Vm43/St10 in the *Chianti Classico* area. Firstly, such BNp type was never detected before in Tuscany (no studies were previously carried out) but it was probably present in that ecosystem at least since 1997, when the Sangiovese vineyard was planted, and it co-evolved adapting to grapevines and other hosts. This hypothesis could be supported by the *dN*/*dS* values of both *vmp1* (2.482, $P = 0.014$) and *stamp* (2.368, $P = 0.019$) genes of BNp strain populations identified in the examined vineyard. In fact, these values are lower than those reported in previous

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studies for Euro-Mediterranean '*Ca*. P. solani' populations [*vmp1* (4.637, P = 0.000) and *stamp* (2.711, P = 0.004)] (Quaglino et al. 2016), possibly indicating a less intensive selection (higher level of adaptation) acting on BNp strains identified in Sangiovese vineyard. Secondly, BNp *vmp1*/*stamp* type Vm43/St10, never reported before, could derive from plant materials and/or insects infected by '*Ca*. P. solani' strains harbouring sequence variant Vm43 (present in Central Italy) and St10 (present in France), separately. Such strains, co-infecting the same host(s), may have underwent a recombination process leading to the appearance of this new BNp type in *Chianti Classico* area. A similar recombination phenomenon was previously proposed for phytoplasmas of taxonomic group 16SrX (Danet et al. 2011).

Interestingly, in both years, Sangiovese clone grapevines exhibited a range of symptom severity, described by a symptomatic scale from zero to three as proposed by Riedle-Bauer et al. (2010) with some modifications. Previous studies indicated that symptom severity of phytoplasma diseases can be influenced by four main factors: (i) environmental conditions (Hren et al. 2009; Murolo and Romanazzi 2015), (ii) phytoplasma abundance in infected plants (Martini et al. 2011), (iii) phytoplasma strain virulence (Seemüller and Schneider 2007; Seemüller et al. 2013), and (iv) plant host genotypes (Bisognin et al. 2008; Roggia et al. 2014; Galetto et al. 2016; Eveillard et al. 2016; Quaglino et al. 2016). Due to the genetic identity among clone Sangiovese grapevine plants and to the homogeneous environmental conditions in each year, the present study focused on investigating the possible correlation between the observed symptom severity and either the abundance and/or genotype of BNp strains.

Statistical analyses showed no significant correlation between relative abundance of BNp phytoplasma and symptom severity. On the other hand, statistically significant differences were observed in the distribution of BNp strains, grouped in *vmp1*, *stamp* and *vmp1*/*stamp* phylogenetic clusters, in grapevine plants showing symptom severity class 1, 2 and 3. Considering the genetic homogeneity of grapevine plants within the examined vineyard, such evidences suggest that the genetic diversity of BNp strains could influence the symptom severity of infected grapevines. In particular, as reported for apple proliferation phytoplasmas (Seemüller and Schneider 2007; Seemüller et al. 2013), BNp strains grouped in distinct phylogenetic clusters could be associated with different range of virulence. Particularly, (i) BNp strains of the cluster *vmp1*/*stamp*-3, present mainly in grapevines showing symptom severity class 3, have the highest average disease severity (A*S*), suggesting a possible high virulence; (ii) BNp strains of the cluster *vmp1*/*stamp*-1, present in grapevines showing symptom severity class 1, 2, and 3, have an intermediate average disease severity and the highest overall disease severity, suggesting a possible moderate virulence. Regarding the prevalent (newly reported) BNp type Vm43/St10, its belonging to the cluster

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vmp1/*stamp*-1, combined with its widespread distribution in the examined vineyard, could lead to hypothesize its co-evolution with Sangiovese clone in the *Chianti Classico* area.

Fascinatingly, nucleotide sequence alignment of *vmp1/stamp* types, identified in the *Chianti Classico* area, revealed the larger inter-cluster genetic diversity of BNp strains (89.41%, about 184 SNPs distinguishing one strain to another) in comparison with the intra-cluster genetic diversity (99.57%, about 10 SNPs distinguishing one strain to another). This evidence can suggest that, within each *vmp1*/*stamp* clusters, BNp strains with extremely similar Vmp1 and Stamp protein sequences could share analogous biological behaviour and functions, leading also to possible differences in their virulence.

The extremely erratic scenario of host-pathogen-environment interactions involved in BN disease can lead to two main reasonable concerns regarding the association between symptom severity and BNp strains: (i) the variation of symptom severity and BNp strain in the same infected plant throughout years, and (ii) the possible co-infection of the same plant by multiple BNp strains. Regarding the former (i), in this work the accuracy of the analysis was improved by considering symptom severity and BNp strain type in the same season, as described in a previous study (Quaglino et al., 2016). Regarding the latter (ii), the BNp strain co-infection in single grapevines was plausibly excluded by checking the collective length of the fragments composing the enzymatic digestion (RFLP) patterns of *vmp1* amplicons. In fact, through RFLP pattern visualization, a multiple phytoplasma strain infection can be detected by the co-presence, in a single digested amplicon, of more than one pattern, overlapped in the same electrophoretic lane. It is easily observed because the sum of the length of the pattern bands is higher than the amplicon size (Alma et al., 1996; Staniulis et al., 2000). In a previous study, the co-infection of grapevine plants by FDp strains was revealed using an approach based on the library analysis of *rplV-rpsC* genes. It evidenced that, while multi-strains infection is frequent, only one strain is strongly prevalent representing over 99% of the population (Quaglino et al., 2010).

Despite the measures used to address these main concerns, further studies are needed to investigate in depth the experimental evidences, collected in the present study, concerning the possible virulence range among BNp strains according to their genetic background, also in other areas affected by 'bois noir'. Moreover, epidemiological patterns of BNp strains, here identified in *Chianti Classico* area, will be monitored in the next years throughout European viticulture regions.

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TABLE 1. Symptom severity class, relative abundance and strain type of BNp identified in symptomatic grapevine plants in Greve in Chianti vineyard over 2015-2016

Plant	2015					2016				
	Symptom	16S rDNA	*vmp1*	Sequence variant		Symptom	16S rDNA	*vmp1*	Sequence variant	
	severity	ΔCt	RFLP type	*vmp1*	*stamp*	severity	ΔCt	RFLP type	*vmp1*	*stamp*
San1	3	7	-	-	-	3	6.08	V11	-	-
San2	3	7.5	V12	vmFi2	stFi3	3	6.54	V12	vmFi2	stFi3
San3	1	4.5	-	-	stFi5	0	-	-	-	-
San4	1	10	-	-	stFi6	2	6.95	V11	-	stFi1
San5	2	6.5	V11	vmFi1	stFi1	3	6.69	V11	vmFi10	stFi1
San6	2	6.5	-	-	stFi7	2	7.53	V12	-	stFi10
San7	1	9	V11	vmFi1	stFi1	1	5.83	V11	vmFi1	stFi1
San8	3	5.5	-	-	stFi2	3	7.17	V12	-	stFi11
San9	3	8	V12	-	stFi2	Eradicated	-	-	-	-
San10	2	8.5	-	-	stFi8	3	7.38	V12	-	stFi2
San11	2	7	V12	vmFi2	stFi3	3	4.97	V12	vmFi8	stFi3
San12	1	6	V12	-	stFi2	0	-	-	-	-
San13	1	10.5	V12	-	stFi2	1	2.12	-	-	-
San14	3	7	-	-	stFi2	2	8.07	V12	-	stFi2
San15	3	8.5	V11	-	stFi1	1	10.41	V11	-	-
San16	3	7	V11	vmFi1	stFi1	3	6.27	V11	vmFi1	stFi12
San17	2	8	V11	vmFi1	stFi1	1	7.68	V11	vmFi1	stFi13
San18	1	11	V12	-	stFi3	0	-	-	-	-
San19	1	8	V12	-	stFi2	2	1.04	-	-	stFi2
San20	2	6	-	-	-	3	7.66	-	-	stFi3
San21	3	6.5	V11	vmFi1	stFi1	3	7.18	V11	vmFi1	stFi1
San22	2	6.5	-	-	stFi9	1	6.73	V11	-	stFi1
San23	2	7	V12	vmFi3	stFi2	3	5.01	V12	vmFi3	stFi2
San24	2	6.5	V12	vmFi4	stFi4	2	5.64	V12	vmFi3	stFi4

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TABLE 2. Symptom severity class, relative abundance and strain type of BNp identified in symptomatic grapevine plants in Greve in Chianti vineyard in 2016

Plant	2016				
	Symptom severity	16S rDNA ΔCt	*vmp1* RFLP type	Sequence variant *vmp1*	Sequence variant *stamp*
San25	3	5.6	V11	vmFi1	stFi1
San26	1	-	-	-	-
San27	1	-	-	-	-
San28	2	5.58	V11	vmFi1	stFi14
San29	3	4.88	-	-	stFi15
San30	2	-	-	-	-
San31	3	8.02	V9	vmFi5	stFi2
San32	1	-	V12	vmFi12	stFi3
San33	3	4.9	V11	-	stFi2
San34	3	10.34	V11	vmFi1	stFi1
San35	1	-	V12	vmFi12	stFi2
San36	2	6.9	V9	vmFi5	stFi2
San37	3	7.13	V9	vmFi9	stFi2
San38	1	6.08	V11	-	stFi1
San39	3	7.1	V12	-	stFi3
San40	3	6.09	V11	vmFi1	stFi1
San41	1	10.06	-	-	-
San42	2	7.59	V11	vmFi1	stFi1
San43	3	4.79	V12	vmFi12	stFi3
San44	1	-	-	-	-
San45	2	5.47	-	-	stFi16
San46	3	5.16	V11	vmFi1	stFi1
San47	2	4.97	V11	vmFi6	stFi2
San48	1	-	-	-	-
San49	3	8.61	V12	vmFi7	stFi3
San50	2	6.88	V11	vmFi1	stFi1
San51	3	5.82	V11	vmFi1	stFi1
San52	2	5.68	V11	vmFi6	stFi2
San53	2	8.79	V11	vmFi1	stFi1
San54	3	2.59	V12	vmFi7	stFi3
San55	3	-	V12	vmFi3	stFi2
San56	3	6.42	V11	vmFi11	stFi1
San57	1	4.56	V11	vmFi1	stFi1

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TABLE 3. *vmp1* genetic variants identified among BNp strains identified in the vineyard in Greve in Chianti over 2015-2016: prevalence, representative strains and sequence accession numbers

vmp1 variant	No. of strains		Representative strain[1]	Accession No.[2]
	2015	2016		
vmFi1 (Vm43)	5	14	San21_2015	MF182856
vmFi2 (Vm45)	2	1	San2_2015	MF182857
vmFi3 (Vm42)	1	3	San24_2016	MF182861
vmFi4	1	0	San24_2015	MF182858
vmFi5 (Vm39)	0	2	San31_2016	MF182859
vmFi6 (Vm41)	0	2	San47_2016	MF182860
vmFi7	0	2	San49_2016	MF182862
vmFi8	0	1	San11_2016	MF182863
vmFi9	0	1	San37_2016	MF182864
vmFi10	0	1	San5_2016	MF182865
vmFi11	0	1	San56_2016	MF182866
vmFi12	0	3	San43_2016	MF182867

[1]The name of each representative strain is composed by plant (as indicated in Table 1 and 2) and year of sampling, separated by an underscore

[2]Accession No. linked to sequences deposited to NCBI GenBank

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TABLE 4. *stamp* genetic variants identified among BNp strains identified in the vineyard in Greve in Chianti over 2015-2016: prevalence, representative strains and sequence accession numbers

stamp variant	No. of strains		Representative strain[1]	Accession No.[2]
	2015	2016		
stFi1 (St10)	5	16	San21_2015	MF182868
stFi2 (St5)	7	12	San23_2015	MF182869
stFi3 (St18)	3	8	San2_2015	MF182870
stFi4 (St22)	1	1	San24_2015	MF182871
stFi5	1	0	San3_2015	MF182872
stFi6	1	0	San4_2015	MF182873
stFi7	1	0	San6_2015	MF182874
stFi8	1	0	San10_2015	MF182875
stFi9	1	0	San22_2015	MF182876
stFi10	0	1	San6_2016	MF182877
stFi11	0	1	San8_2016	MF182878
stFi12	0	1	San16_2016	MF182879
stFi13	0	1	San17_2016	MF182880
stFi14	0	1	San28_2016	MF182881
stFi15	0	1	San29_2016	MF182882
stFi16	0	1	San45_2016	MF182883

[1]The name of each representative strain is composed by plant (as indicated in Table 1 and 2) and year of sampling, separated by an underscore

[2]Accession No. linked to sequences deposited to NCBI GenBank

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TABLE 5. Genetic diversity among *vmp1*/*stamp* types, identified in *Chianti Classico* area, grouped in the same and in distinct phylogenetic clusters

vmp1/*stamp* cluster	type	#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	vmFi1/stFi13	**1**	ID																
	vmFi1/stFi14	**2**	0,998	ID															
	vmFi1/stFi12	**3**	0,998	0,998	ID														
	vmFi1/stFi1	**4**	0,999	0,998	0,999	ID													
	vmFi10/stFi1	**5**	0,998	0,997	0,998	0,998	ID												
	vmFi11/stFi1	**6**	0,998	0,998	0,998	0,999	0,999	ID											
3	vmFi2/stFi3	**7**	0,831	0,831	0,831	0,832	0,831	0,832	ID										
	vmFi8/stFi3	**8**	0,832	0,831	0,832	0,832	0,832	0,832	0,999	ID									
	vmFi12/stFi3	**9**	0,831	0,831	0,831	0,832	0,831	0,832	0,999	0,998	ID								
	vmFi12/stFi2	**10**	0,832	0,831	0,832	0,832	0,832	0,832	0,99	0,99	0,991	ID							
4	vmFi7/stFi3	**11**	0,944	0,943	0,944	0,945	0,944	0,945	0,879	0,878	0,878	0,87	ID						
	vmFi6/stFi2	**12**	0,948	0,948	0,948	0,949	0,948	0,949	0,868	0,868	0,868	0,876	0,986	ID					
	vmFi3/stFi2	**13**	0,948	0,948	0,948	0,949	0,948	0,949	0,867	0,867	0,867	0,875	0,985	0,998	ID				
	vmFi4/stFi4	**14**	0,945	0,944	0,945	0,945	0,945	0,945	0,869	0,869	0,869	0,877	0,988	0,994	0,996	ID			
	vmFi3/stFi4	**15**	0,948	0,947	0,948	0,948	0,948	0,948	0,867	0,866	0,866	0,875	0,984	0,998	0,999	0,996	ID		
5	vmFi5/stFi2	**16**	0,928	0,928	0,928	0,929	0,928	0,928	0,825	0,824	0,825	0,833	0,937	0,95	0,949	0,945	0,948	ID	
	vmFi9/stFi2	**17**	0,928	0,927	0,928	0,928	0,927	0,928	0,825	0,825	0,825	0,834	0,937	0,951	0,949	0,946	0,949	0,999	ID

Mean % sequence identity within clusters: *vmp1*/*stamp*-1: 99.82; *vmp1*/*stamp*-3: 99.45; *vmp1*/*stamp*-4: 99.24; *vmp1*/*stamp*-5: 99.9

Mean % sequence identity among clusters: *vmp1*/*stamp*-1 vs *vmp1*/*stamp*-3: 83.15; *vmp1*/*stamp*-1 vs *vmp1*/*stamp*-4: 94.67; *vmp1*/*stamp*-1 vs *vmp1*/*stamp*-5: 92.76; *vmp1*/*stamp*-3 vs *vmp1*/*stamp*-4: 87.09; *vmp1*/*stamp*-3 vs *vmp1*/*stamp*-5: 82.7; *vmp1*/*stamp*-4 vs *vmp1*/*stamp*-5: 94.61

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Figure legends

Fig. 1. Virtual *Rsa*I-RFLP profiles of *vmp1* gene obtained from BNp strains detected in the study vineyard in 2015 and 2016. Virtual *Rsa*I restriction profiles of *vmp1* gene were obtained digesting trimmed TYPH10F/TYPH10R fragments with the software pDRAW32. San21_2015, representative of 30 BNp strains exhibiting the *Rsa*I-RFLP profile of V11; San2_2015, representative of 24 BNp strains exhibiting the *Rsa*I-RFLP profile of V12; San31_2016, representative of 3 BNp strains exhibiting the *Rsa*I-RFLP profile of V9; M, Marker Φ174 DNA/*Hae*III (Promega), fragment length from top to bottom: 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72 bp.

Fig. 2. Phylogenetic position of *vmp1* gene sequence variants identified among BNp strains in the *Chianti Classico* area and their relationship with symptom and disease severity in Sangiovese grapevines. (**A**) Unrooted phylogenetic tree inferred from *vmp1* gene nucleotide sequences of '*Ca.* Phytoplasma solani' strains representing *vmp1* sequence variants in GenBank (Supplementary Table S1) and identified in the *Chianti Classico* area (written in bold); *vmp1* gene sequences minimum evolution analysis was performed using the neighbor-joining method and bootstrap replicated 1.000 times. Name of strains are reported on the image; (**B**) Graphic representing the distribution of symptom severity classes, registered in 2016 in the study vineyard, in association with BNp strains grouped according to *vmp1* phylogenetic clusters; significantly different distributions (χ^2 test, $p < 0.1$) are indicated with * at the top of each bar; A*S* (average disease severity) indicates the average severity of symptoms shown by grapevine plants infected by BNp strains grouped according to *vmp1* phylogenetic clusters, O*S*% (overall disease severity %) indicates how much each BNp strains cluster contributes to the severity of the disease observed in the study vineyard in 2016.

Fig. 3. Phylogenetic position of *stamp* gene sequence variants identified among BNp strains in the *Chianti Classico* area and their relationship with symptom and disease severity in Sangiovese grapevines. (**A**) Unrooted phylogenetic tree inferred from *stamp* gene nucleotide sequences of '*Ca.* Phytoplasma solani' strains representing *stamp* sequence variants in GenBank (Supplementary Table S4) and identified in the *Chianti Classico* area (written in bold); minimum evolution analysis was performed using the neighbor-joining method and bootstrap replicated 1.000 times. Name of strains are reported on the image; (**B**) Graphic representing the distribution of symptom severity classes, registered in 2016 in the study vineyard, in association with BNp strains grouped according to *stamp* phylogenetic clusters; significantly different distributions (χ^2 test, $p < 0.1$) are indicated with * at the top of each bar; A*S* (average disease severity) indicates the average severity of

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symptoms shown by grapevine plants infected by BNp strains grouped according to *stamp* phylogenetic clusters, O*S*% (overall disease severity %) indicates how much each BNp strains cluster contributes to the severity of the disease observed in the vineyard in 2016.

Fig. 4. Phylogenetic position of *vmp1*/*stamp* types identified among BNp strains in the *Chianti Classico* area and their relationship with symptom and disease severity in Sangiovese grapevines. (**A**) Unrooted phylogenetic tree inferred from *vmp1* and *stamp* gene concatenated nucleotide sequences of '*Ca.* Phytoplasma solani' strains representing *vmp1*/*stamp* types in GenBank (Supplementary Table S6) and identified in the *Chianti Classico* area (written in bold); minimum evolution analysis was performed using the neighbor-joining method and bootstrap replicated 1.000 times. Name of strains are reported on the image; (**B**) Graphic representing the distribution of symptom severity classes, registered in 2016 in the study vineyard, in association with BNp strains grouped according to *vmp1*/*stamp* phylogenetic clusters; significantly different distributions (χ^2 test, $p < 0.1$) are indicated with * at the top of each bar; A*S* (average disease severity) indicates the average severity of symptoms shown by grapevine plants infected by BNp strains grouped according to *vmp1*/*stamp* phylogenetic clusters, O*S*% (overall disease severity %) indicates how much each BNp strains cluster contributes to the severity of the disease observed in the study vineyard in 2016.

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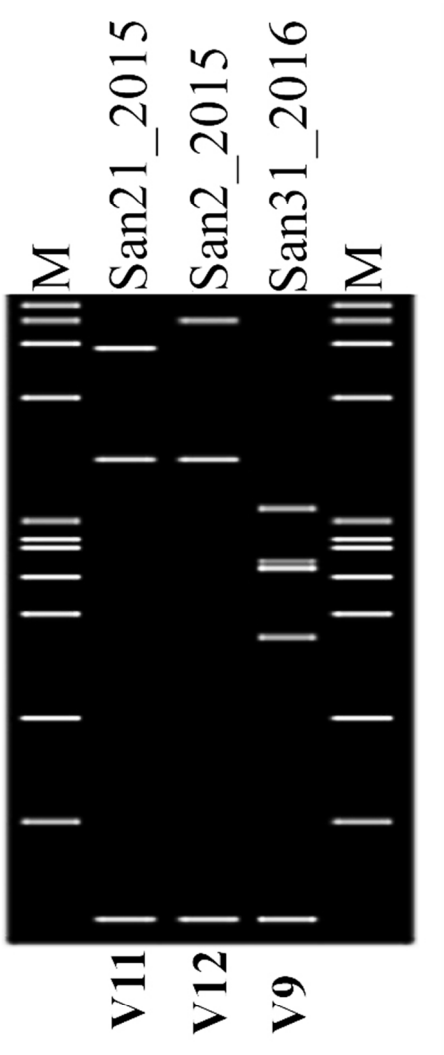

Figure 1

Fig. 1. Virtual *Rsa*I-RFLP profiles of *vmp1* gene obtained from BNp strains detected in the study vineyard in 2015 and 2016. Virtual *Rsa*I restriction profiles of *vmp1* gene were obtained digesting trimmed TYPH10F/TYPH10R fragments with the software pDRAW32. San21_2015, representative of 30 BNp strains exhibiting the *Rsa*I-RFLP profile of V11; San2_2015, representative of 24 BNp strains exhibiting the *Rsa*I-RFLP profile of V12; San31_2016, representative of 3 BNp strains exhibiting the *Rsa*I-RFLP profile of V9; M, Marker Φ174 DNA/*Hae*III (Promega), fragment length from top to bottom: 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72 bp.

37x92mm (300 x 300 DPI)

Figure 2

Fig. 2. Phylogenetic position of *vmp1* gene sequence variants identified among BNp strains in the *Chianti Classico* area and their relationship with symptom and disease severity in Sangiovese grapevines. (**A**) Unrooted phylogenetic tree inferred from *vmp1* gene nucleotide sequences of '*Ca*. Phytoplasma solani' strains representing *vmp1* sequence variants in GenBank (Supplementary Table S1) and identified in the *Chianti Classico* area (written in bold); *vmp1* gene sequences minimum evolution analysis was performed using the neighbor-joining method and bootstrap replicated 1.000 times. Name of strains are reported on the image; (**B**) Graphic representing the distribution of symptom severity classes, registered in 2016 in the study vineyard, in association with BNp strains grouped according to *vmp1* phylogenetic clusters; significantly different distributions (χ^2 test, $p < 0.1$) are indicated with * at the top of each bar; A*S* (average disease severity) indicates the average severity of symptoms shown by grapevine plants infected by BNp strains grouped according to *vmp1* phylogenetic clusters, O*S*% (overall disease severity %) indicates how much each BNp strains cluster contributes to the severity of the disease observed in the study vineyard in 2016.

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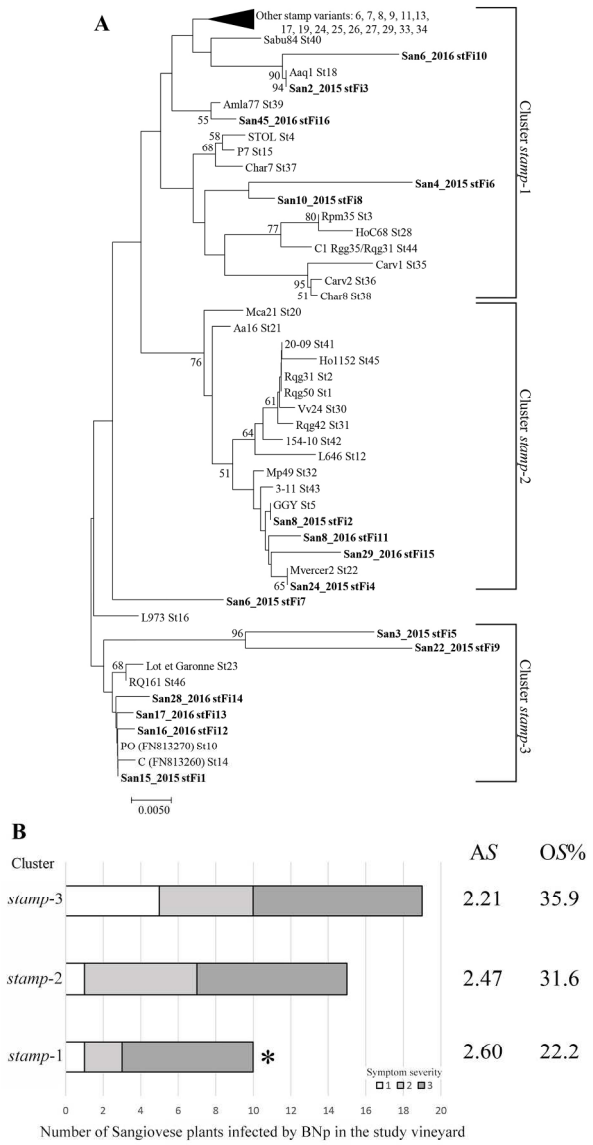

Figure 3

Fig. 3. Phylogenetic position of *stamp* gene sequence variants identified among BNp strains in the *Chianti Classico* area and their relationship with symptom and disease severity in Sangiovese grapevines. (**A**) Unrooted phylogenetic tree inferred from *stamp* gene nucleotide sequences of '*Ca*. Phytoplasma solani' strains representing *stamp* sequence variants in GenBank (Supplementary Table S4) and identified in the *Chianti Classico* area (written in bold); minimum evolution analysis was performed using the neighbor-joining method and bootstrap replicated 1.000 times. Name of strains are reported on the image; (**B**) Graphic representing the distribution of symptom severity classes, registered in 2016 in the study vineyard, in association with BNp strains grouped according to *stamp* phylogenetic clusters; significantly different distributions (χ^2 test, $p < 0.1$) are indicated with * at the top of each bar; A*S* (average disease severity) indicates the average severity of symptoms shown by grapevine plants infected by BNp strains grouped according to *stamp* phylogenetic clusters, O*S*% (overall disease severity %) indicates how much each BNp strains cluster contributes to the severity of the disease observed in the vineyard in 2016.

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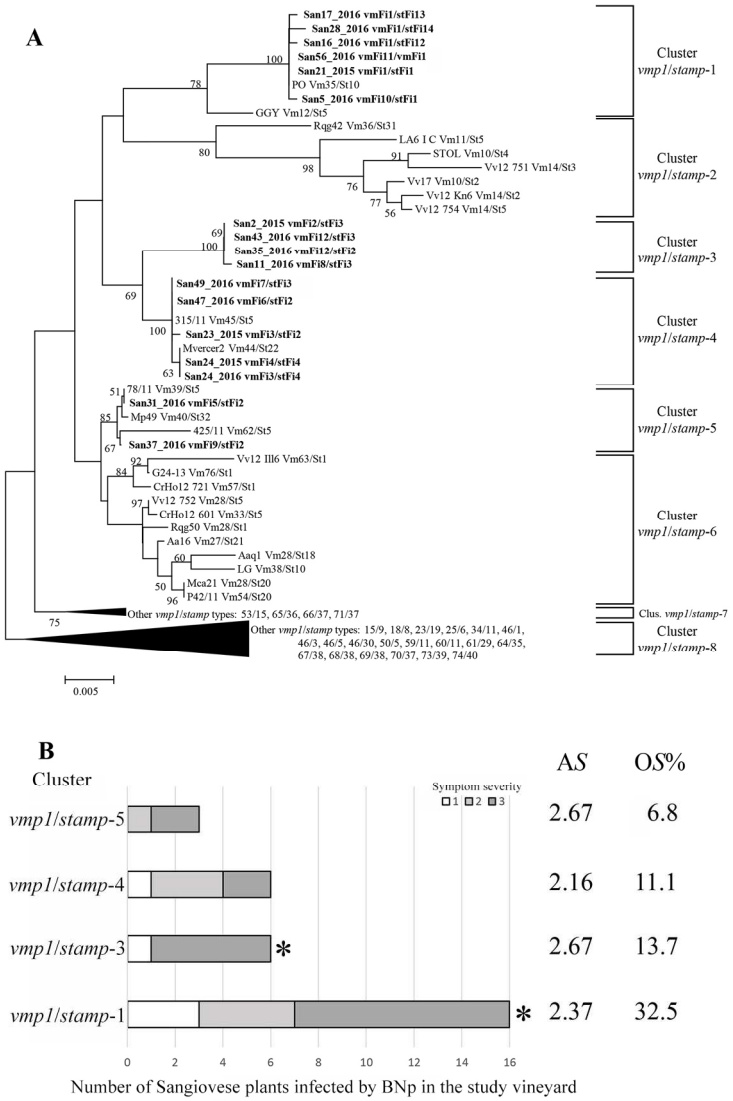

Figure 4

Fig. 4. Phylogenetic position of *vmp1/stamp* types identified among BNp strains in the *Chianti Classico* area and their relationship with symptom and disease severity in Sangiovese grapevines. (**A**) Unrooted phylogenetic tree inferred from *vmp1* and *stamp* gene concatenated nucleotide sequences of '*Ca*. Phytoplasma solani' strains representing *vmp1/stamp* types in GenBank (Supplementary Table S6) and identified in the *Chianti Classico* area (written in bold); minimum evolution analysis was performed using the neighbor-joining method and bootstrap replicated 1.000 times. Name of strains are reported on the image; (**B**) Graphic representing the distribution of symptom severity classes, registered in 2016 in the study vineyard, in association with BNp strains grouped according to *vmp1/stamp* phylogenetic clusters; significantly different distributions (χ^2 test, $p < 0.1$) are indicated with * at the top of each bar; AS(average disease severity) indicates the average severity of symptoms shown by grapevine plants infected by BNp strains grouped according to *vmp1/stamp* phylogenetic clusters, OS% (overall disease severity %) indicates how much each BNp strains cluster contributes to the severity of the disease observed in the study vineyard in 2016.

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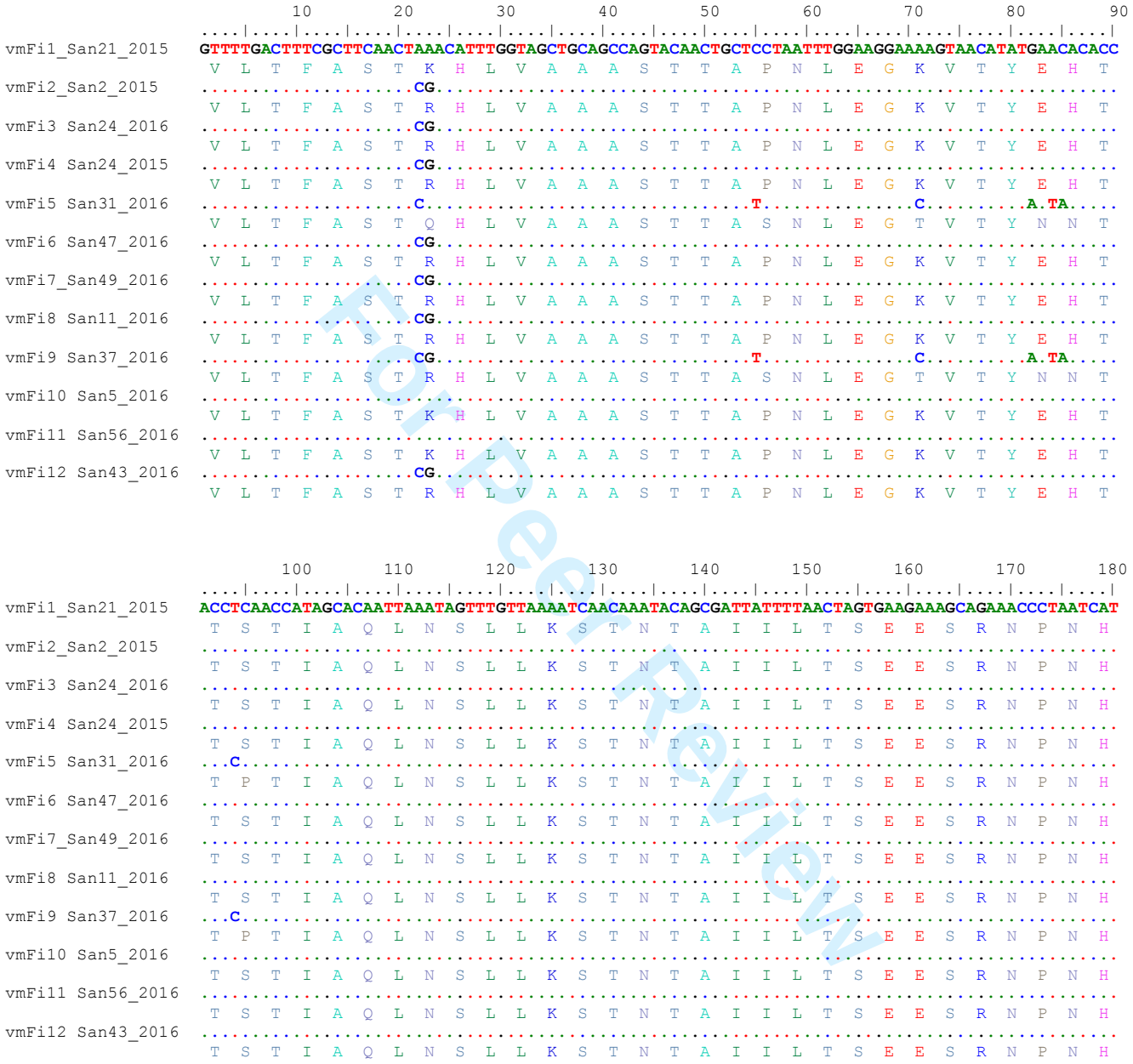
SUPPORTING MATERIAL (Pierro et al. - Phytopathology)

Figure S1. Alignment of nucleotide and *in silico* translated amino acid protein sequences of *vmp1* gene sequence variants identified in the *Chianti Classico* area



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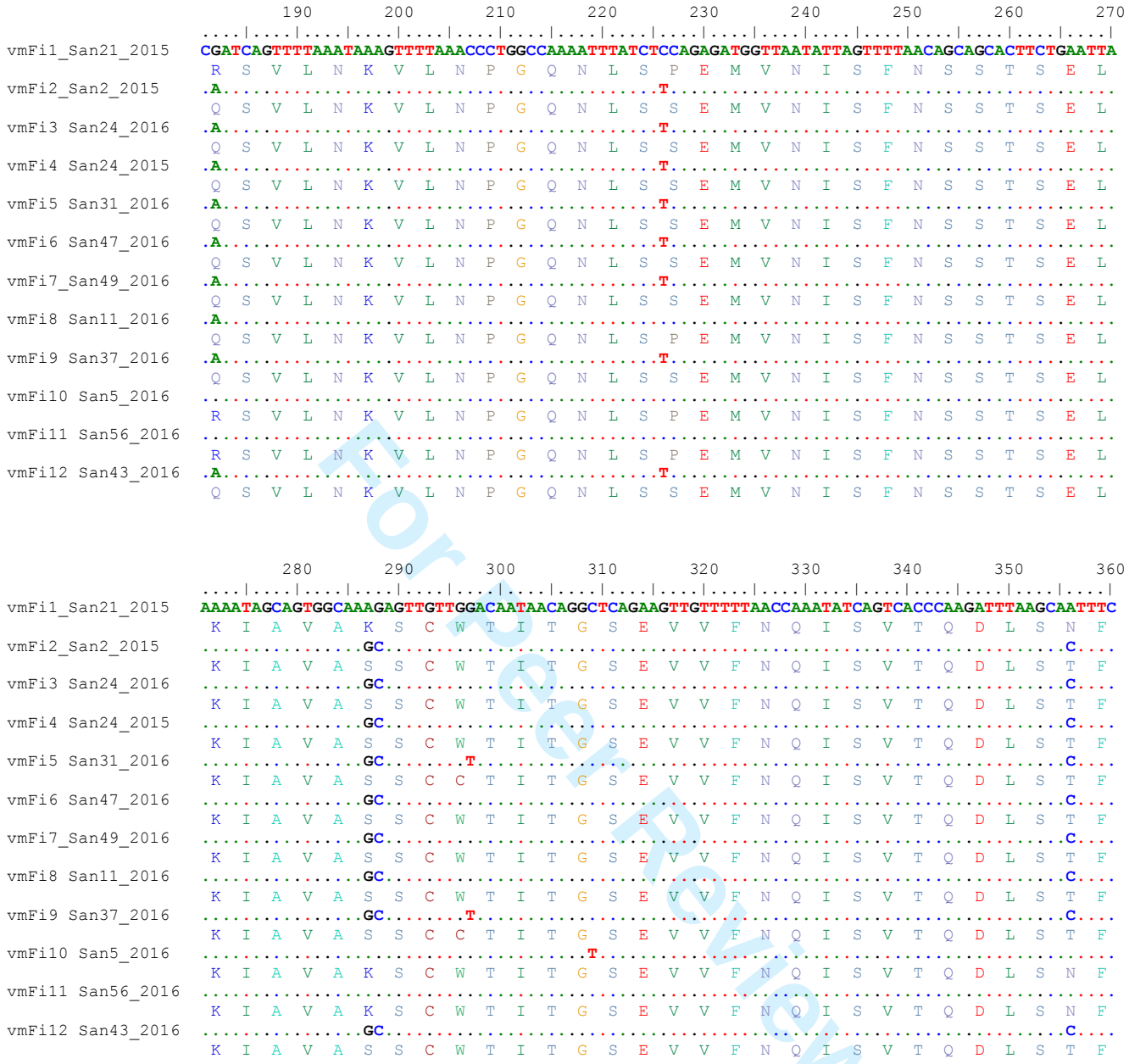

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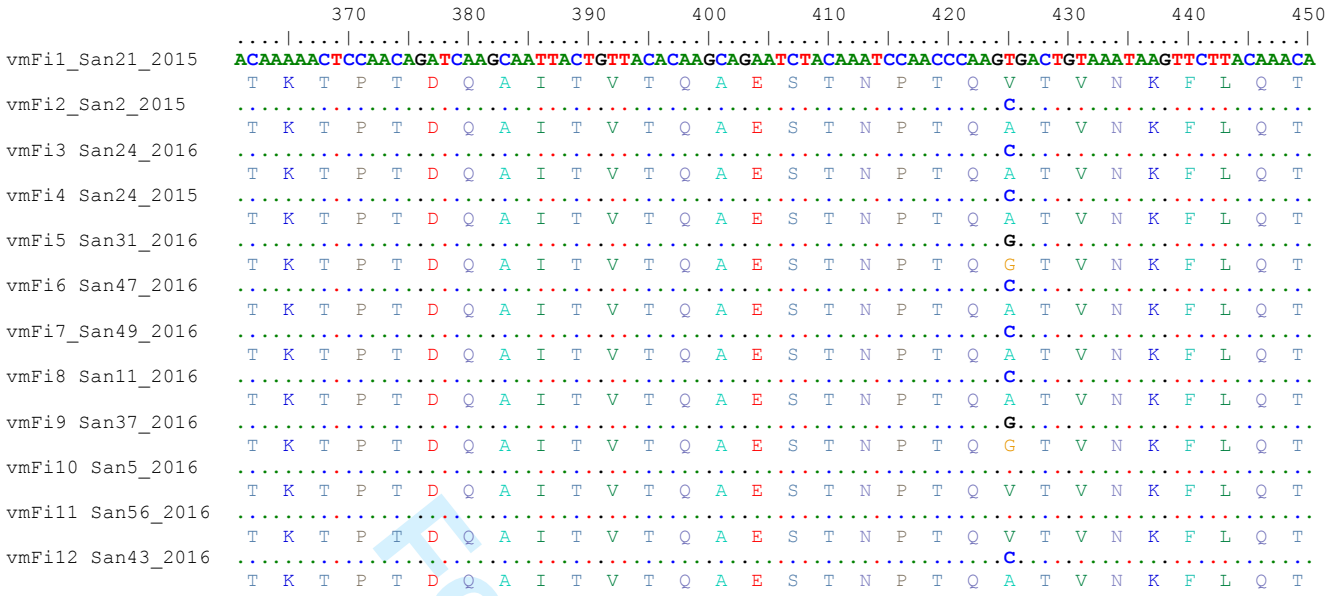

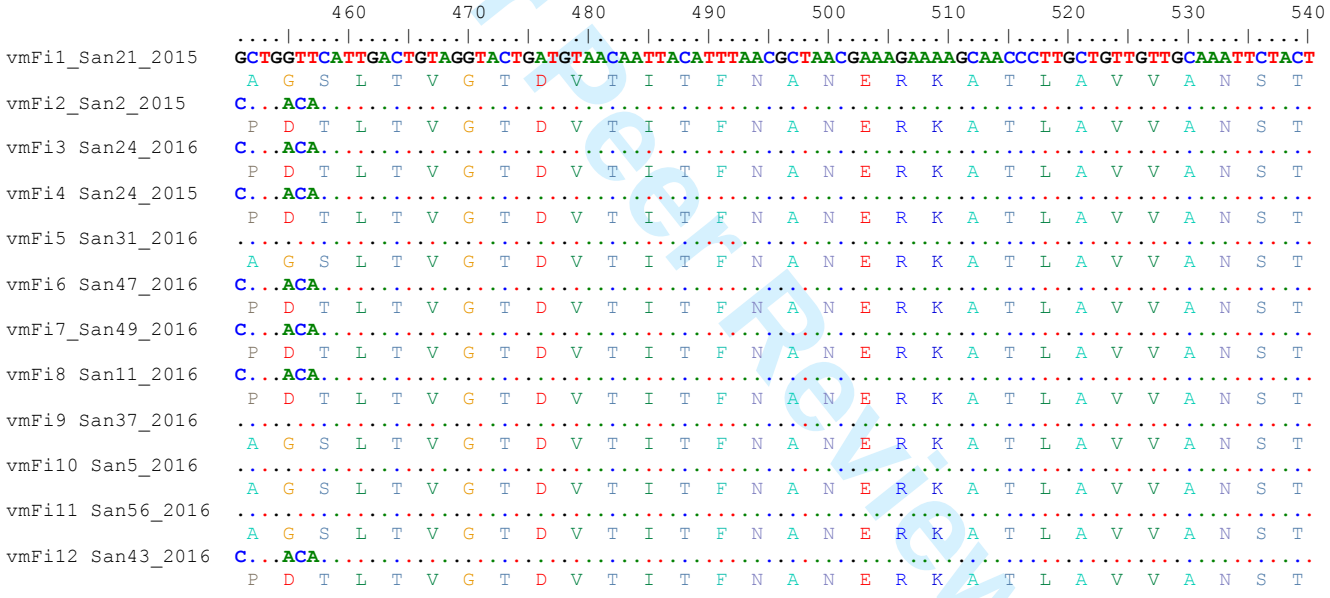

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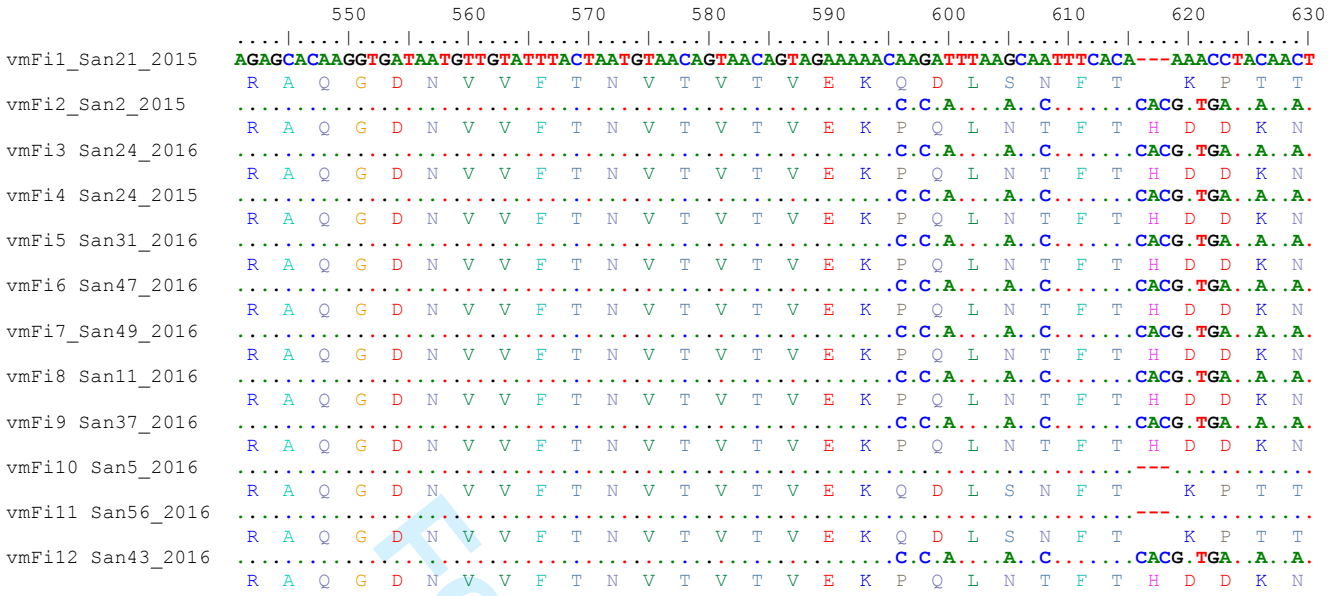

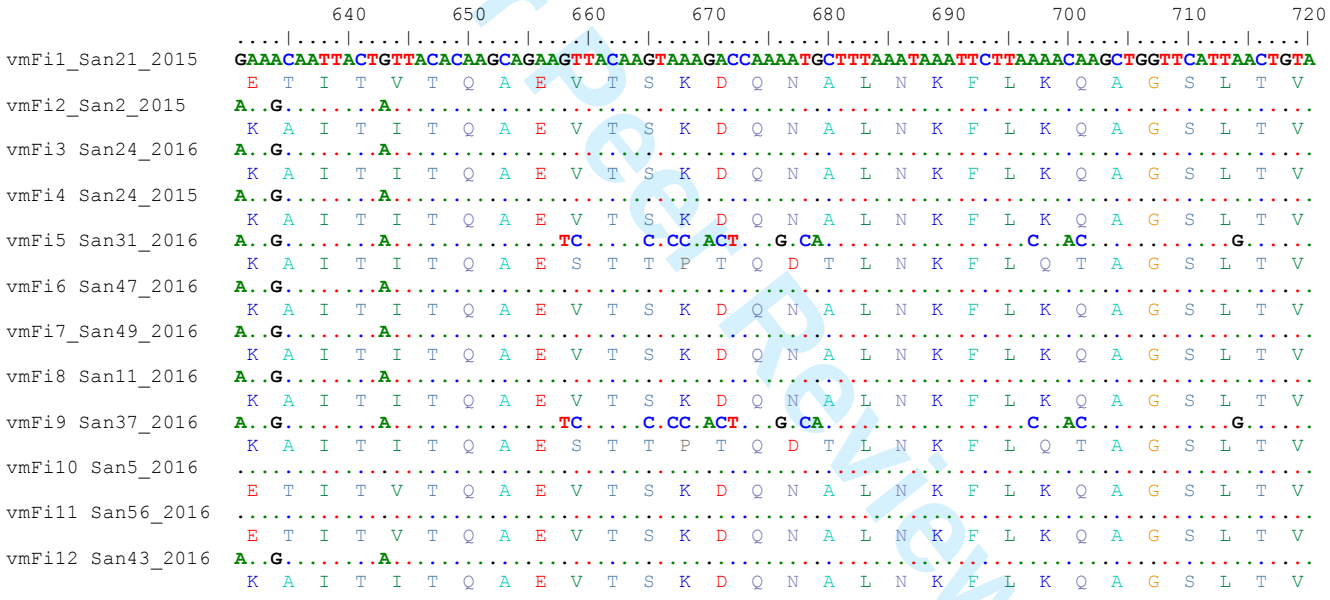

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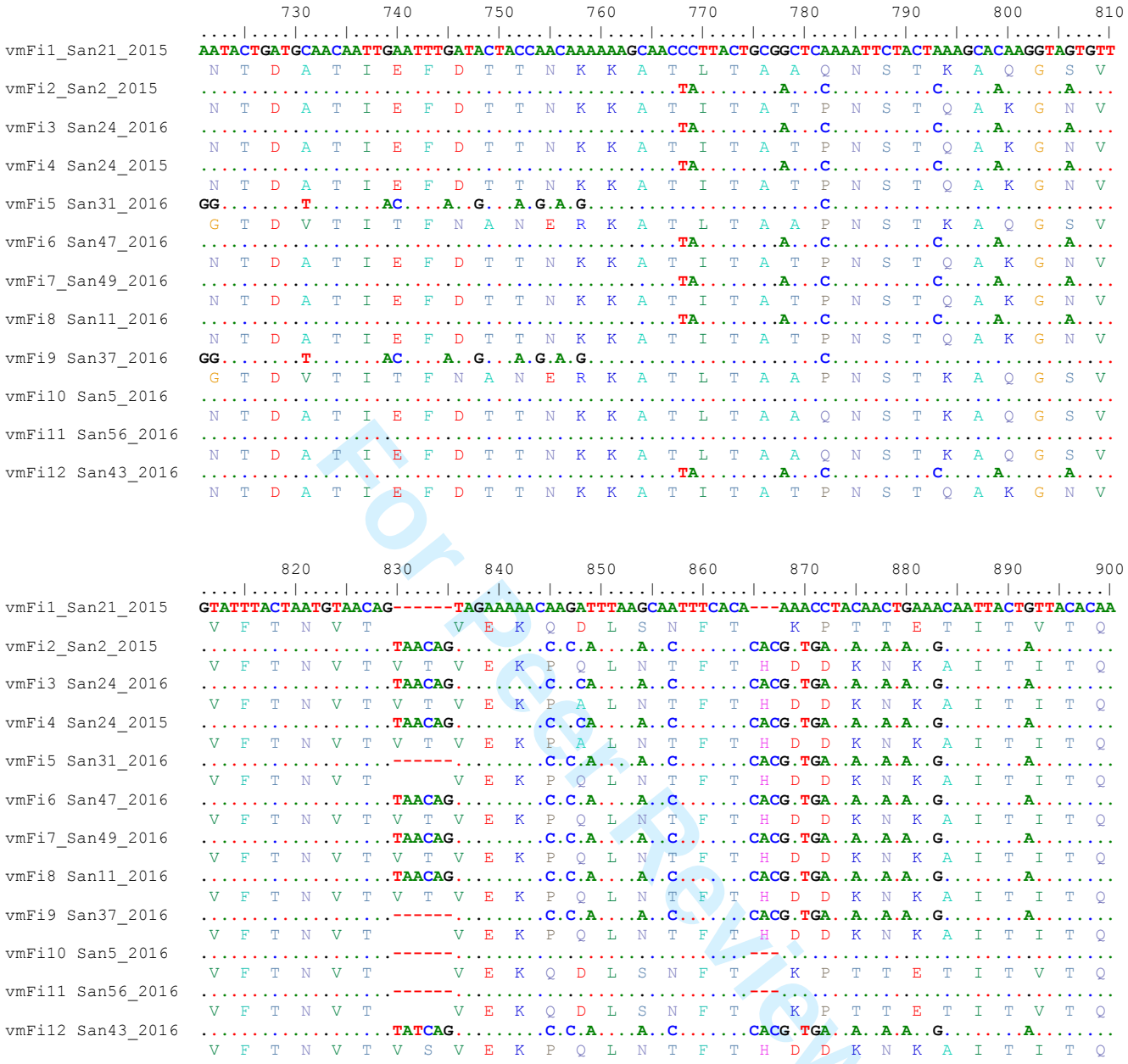

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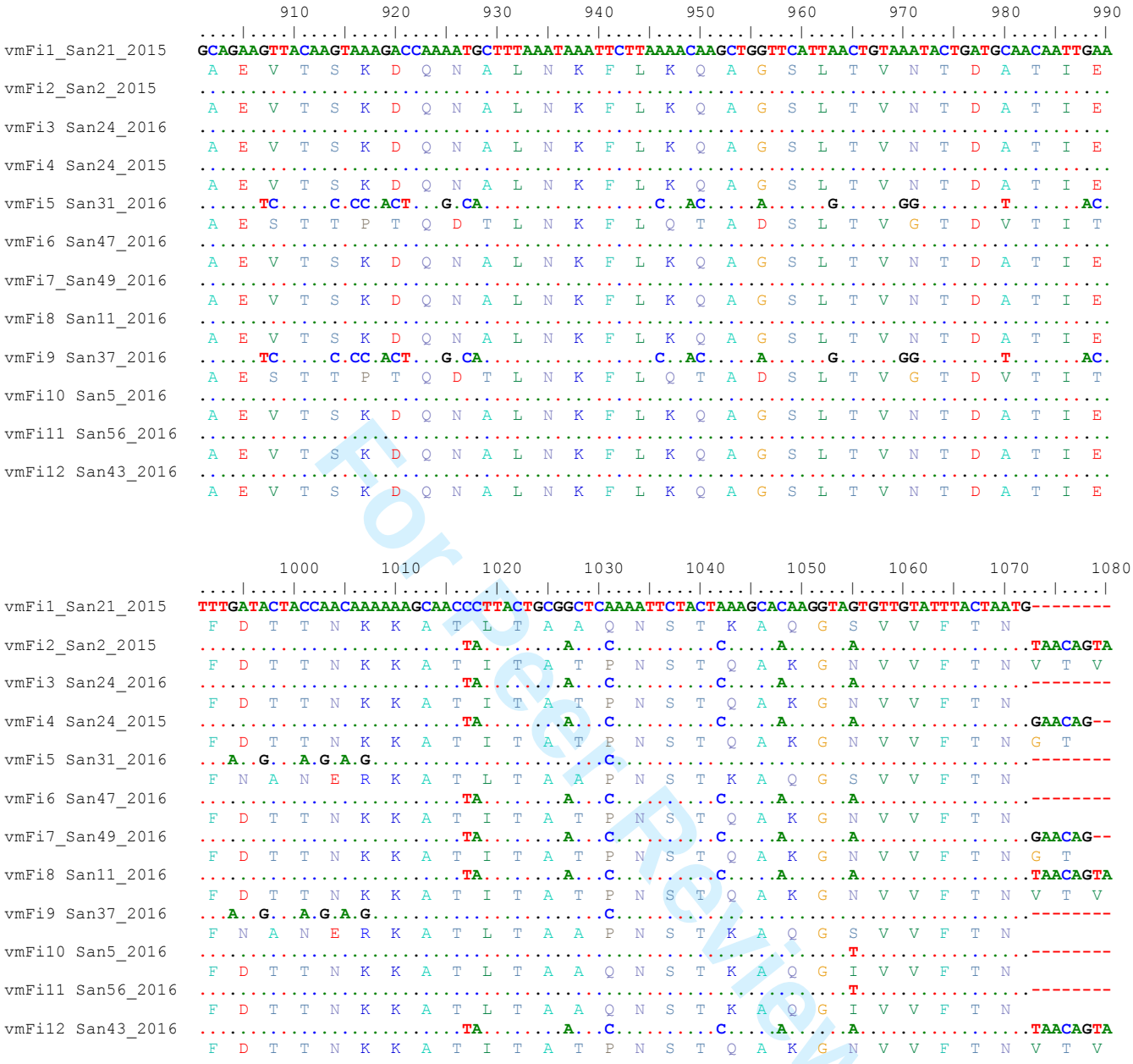

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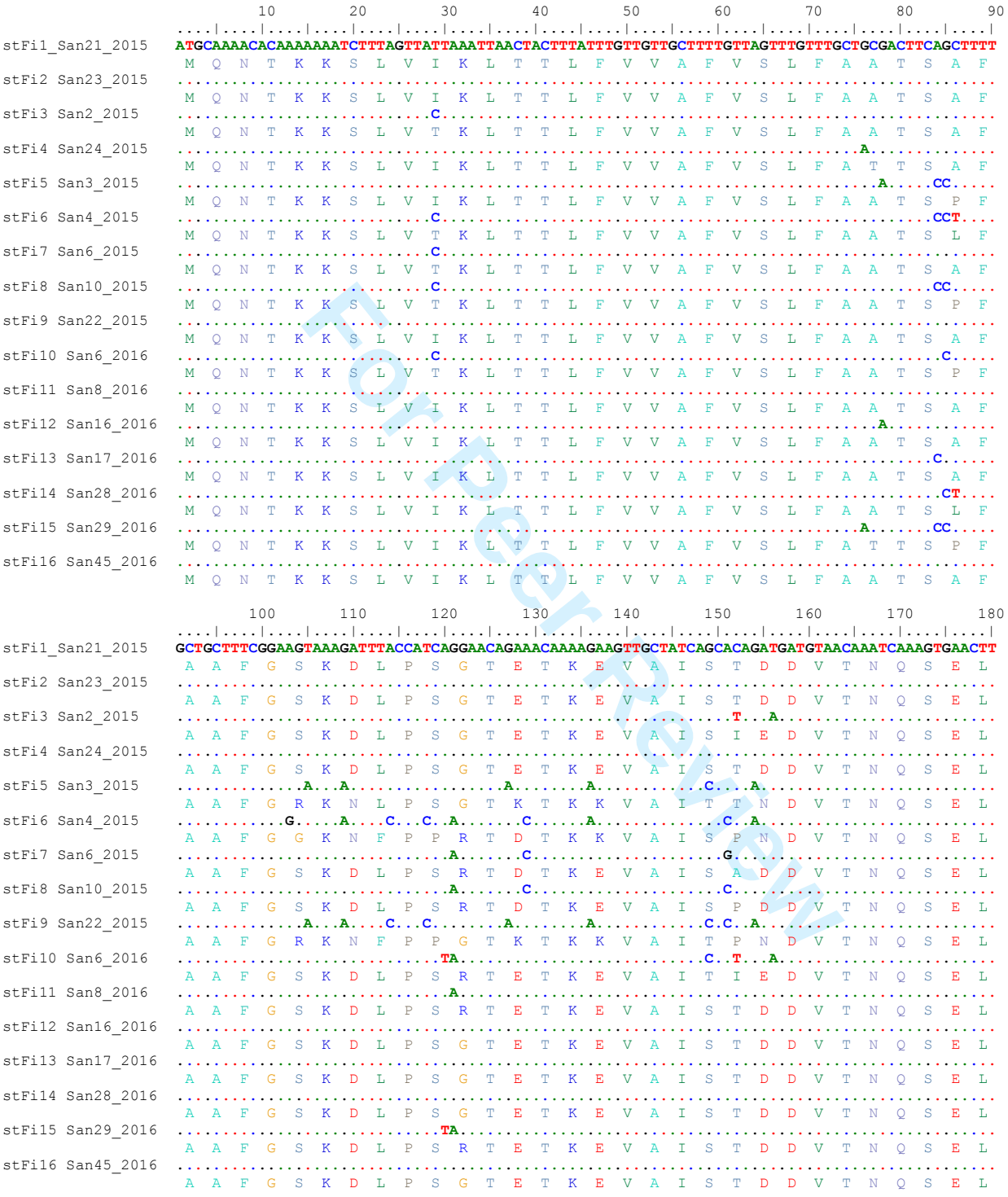
SUPPORTING MATERIAL (Pierro et al. - Phytopathology)

Figure S2. Alignment of nucleotide and *in silico* translated amino acid protein sequences of *stamp* gene sequence variants identified in the *Chianti Classico* area



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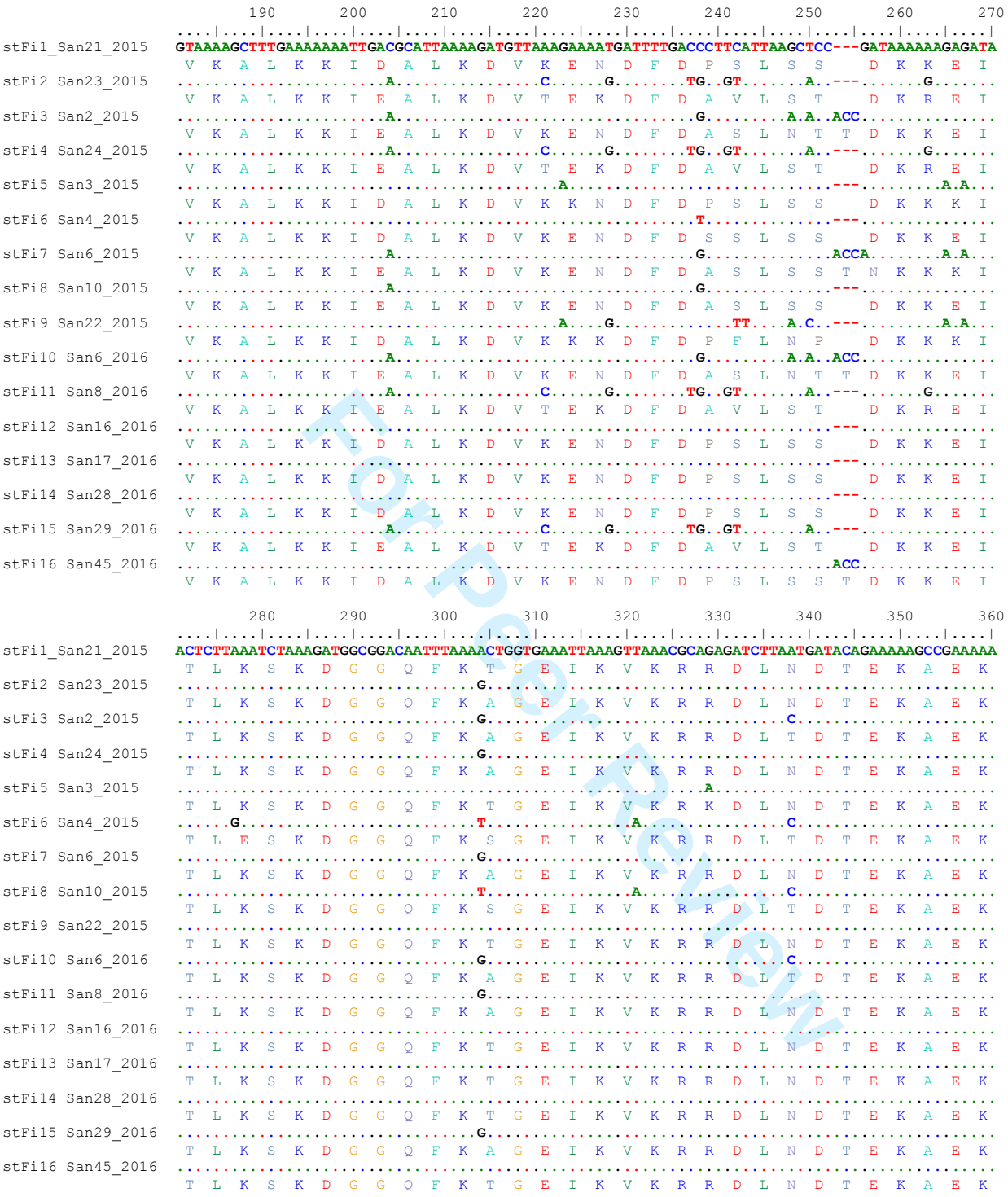

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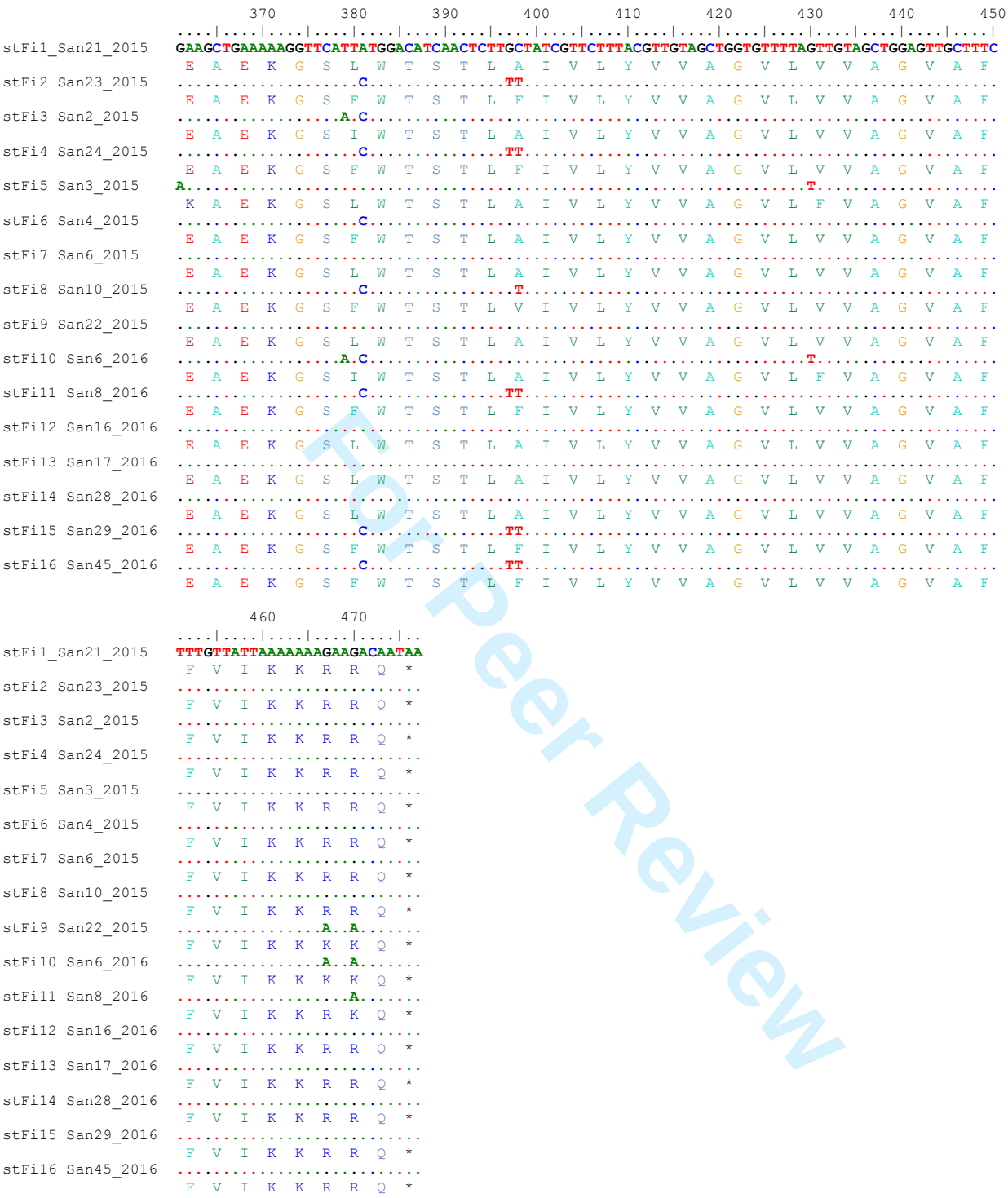

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SUPPORTING MATERIAL (Pierro et al. - Phytopathology)

Table S1. Sequence variants of the gene *vmp1* among '*Ca*. P. solani' strains available in GenBank (part I)

Sequence variant	Strain	Host	Location	Acc. No. *vmp1*
Vm1	S13	*Vitis vinifera*	Italy	HM008616
Vm2	Neuweier57_C	*Convolvulus arvensis*	Germany	JQ977736
Vm3	C_Boppard_C	*Convolvulus arvensis*	Germany	JQ977734
Vm4	N10	*Urtica dioica*	France	JQ977730
Vm5	N9	*Urtica dioica*	France	JQ977729
Vm6	N2	*Urtica dioica*	Germany	JQ977722
Vm7	N1	*Urtica dioica*	Germany	JQ977721
Vm8	CH1	*Vitis vinifera*	Italy	AM992105
Vm8	T2_56	*Solanum lycopersicum*	Italy	AM992104
Vm9	P74/11	*Vitis vinifera*	Italy	KJ145361
Vm10	Vv17	*Vitis vinifera*	Serbia	KC703032
Vm10	Vv21	*Vitis vinifera*	Serbia	KC703026
Vm10	Vexp Rpm5	*Reptalus panzeri*	Serbia	KC703028
Vm10	Vexp Rpg11	*Reptalus panzeri*	Serbia	KC703027
Vm10	Rpm34	*Reptalus panzeri*	Serbia	KC703024
Vm10	Rpg39	*Reptalus panzeri*	Serbia	KC703023
Vm10	Rqg31	*Reptalus quinquecostatus*	Serbia	KC703031
Vm10	Rqg60	*Reptalus quinquecostatus*	Serbia	KC703025
Vm10	STOL	*Capsicum annuum*	Serbia	AM992103
Vm11	LA6_I_C	*Convolvulus arvensis*	Germany	JQ977735
Vm12	GGY	*Vitis vinifera*	Germany	AM992102
Vm13	MK29	*Vitis vinifera*	Macedonia	KF957604
Vm14	Vv12_754	*Vitis vinifera*	Austria	KJ469734
Vm14	Vv12_751	*Vitis vinifera*	Austria	KJ469734
Vm14	Vv12_Kn6	*Vitis vinifera*	Austria	KJ469734
Vm15	60/11	*Vitis vinifera*	Italy	KJ145346
Vm15	Aa25	*Vitis vinifera*	Italy	HM008614
Vm15	Mri10	*Vitis vinifera*	Italy	HM008615
Vm15	HY.86N	*Hyalesthes obsoletus*	Italy	KM225871
Vm15	HY.80N	*Hyalesthes obsoletus*	Italy	KM225870
Vm15	Ne.10	*Urtica dioica*	Italy	KM225869
Vm15	Ho13_1006	*Hyalesthes obsoletus*	Austria	KJ469727
Vm16	N13	*Urtica dioica*	Italy	JQ977733
Vm17	N12	*Urtica dioica*	Italy	JQ977732
Vm18	N11	*Urtica dioica*	Italy	JQ977731
Vm18	Ho13_838	*Hyalesthes obsoletus*	Austria	KJ469729
Vm19	N8	*Urtica dioica*	Italy	JQ977728
Vm20	N7	*Urtica dioica*	Italy	JQ977727
Vm21	N6	*Urtica dioica*	Italy	JQ977726
Vm22	N5	*Urtica dioica*	Italy	JQ977725
Vm23	CrHo13_1183	*Hyalesthes obsoletus*	Austria	KJ469728
Vm23	N4	*Urtica dioica*	Slovenia	JQ977724
Vm24	N3	*Urtica dioica*	Slovenia	JQ977723
Vm25	MK44	*Vitis vinifera*	Macedonia	KF957605
Vm26	149/11	*Vitis vinifera*	Italy	KJ145347
Vm27	Aa16	*Vitis vinifera*	Italy	HM008602
Vm27	Bi.15	*Convolvulus arvensis*	Italy	KM225875
Vm27	HY.9B	*Hyalesthes obsoletus*	Italy	KM225874

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Table S1. Sequence variants of the gene *vmp1* among '*Ca*. P. solani' strains available in GenBank (part II)

Sequence variant	Strain	Host	Location	Acc. No. *vmp1*
Vm28	166/11	*Vitis vinifera*	Italy	KJ145355
Vm28	136/11	*Vitis vinifera*	Italy	KJ145354
Vm28	P10/11	*Vitis vinifera*	Italy	KJ145353
Vm28	Aaq1	*Vitis vinifera*	Italy	HM008601
Vm28	Mca21	*Vitis vinifera*	Italy	HM008599
Vm28	B51	*Vitis vinifera*	Italy	HM008600
Vm28	Rpg47	*Reptalus panzeri*	Serbia	KC703034
Vm28	Rqg50	*Reptalus quinquecostatus*	Serbia	KC703033
Vm28	D_Bacharach_C	*Convolvulus arvensis*	Germany	JQ977738
Vm28	MK28	*Vitis vinifera*	Macedonia	KF957603
Vm28	CrAr12_722_2	*Anaceratagallia ribauti*	Austria	KJ469735
Vm28	Vv12_752	*Vitis vinifera*	Austria	KJ469735
Vm28	17-11	*Vitis vinifera*	Bosnia & Herzegovina	KP739858
Vm29	M33_F_C	*Convolvulus arvensis*	France	JQ977742
Vm30	EisHo1_C	*Convolvulus arvensis*	Italy	JQ977740
Vm31	Charente-1	*Hyalesthes obsoletus*	France	AM992098
Vm32	Moliere	*Prunus avium*	France	AM992096
Vm33	CrHo12_601	*Hyalesthes obsoletus*	Austria	KJ469730
Vm34	19-25	*Vitis vinifera*	Germany	AM992101
Vm35	PO	*Hyalesthes obsoletus*	France	AM992095
Vm36	Rqg42	*Reptalus quinquecostatus*	Serbia	KC703030
Vm37	SFRT1	*Vitis vinifera*	Italy	KJ129606
Vm38	LG	*Solanum lycopersicum*	France	AM992097
Vm39	78/11	*Vitis vinifera*	Italy	KJ145349
Vm39	B7	*Vitis vinifera*	Italy	HM008608
Vm39	HY.31B	*Hyalesthes obsoletus*	Italy	KM225862
Vm39	HY.24B	*Hyalesthes obsoletus*	Italy	KM225861
Vm40	Mp49	*Vitis vinifera*	Italy	HM008607
Vm41	B2035	*Vitis vinifera*	Italy	HM008611
Vm41	C1	*Vitis vinifera*	Italy	HM008610
Vm42	Mca28	*Vitis vinifera*	Italy	HM008609
Vm43	ARSIA1	*Linaria vulgaris*	Italy	KJ129605
Vm43	HY.3B	*Hyalesthes obsoletus*	Italy	KM225877
Vm43	HY32.B	*Hyalesthes obsoletus*	Italy	KM225876
Vm44	Mvercer2	*Vitis vinifera*	Italy	HM008612
Vm45	315/11	*Vitis vinifera*	Italy	KJ145360
Vm45	P136/11	*Vitis vinifera*	Italy	KJ145358
Vm45	P75/11	*Vitis vinifera*	Italy	KJ145357
Vm45	411/11	*Vitis vinifera*	Italy	KJ145359
Vm45	Bi.47	*Convolvulus arvensis*	Italy	KM225881
Vm45	HY.48N	*Hyalesthes obsoletus*	Italy	KM225880
Vm45	HY.50B	*Hyalesthes obsoletus*	Italy	KM225879

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Table S1. Sequence variants of the gene *vmp1* among '*Ca*. P. solani' strains available in GenBank (part III)

Sequence variant	Strain	Host	Location	Acc. No. *vmp1*
Vm46	353/11	*Vitis vinifera*	Italy	KJ145352
Vm46	287/11	*Vitis vinifera*	Italy	KJ145351
Vm46	115/11	*Vitis vinifera*	Italy	KJ145350
Vm46	Mp46	*Vitis vinifera*	Italy	HM008606
Vm46	Ag4a	*Vitis vinifera*	Italy	HM008605
Vm46	Bi.13	*Convolvulus arvensis*	Italy	KM225866
Vm46	Vv24	*Vitis vinifera*	Serbia	KC703036
Vm46	Vv5	*Vitis vinifera*	Serbia	KC703035
Vm46	Rpm35	*Reptalus panzeri*	Serbia	KC703029
Vm46	PM1	*Solanum tuberosum*	Montenegro	KU588192
Vm47	B49	*Vitis vinifera*	Italy	HM008604
Vm48	C3	*Vitis vinifera*	Italy	HM008603
Vm49	MK19	*Vitis vinifera*	Macedonia	KF957602
Vm50	HY.14B	*Hyalesthes obsoletus*	Italy	KM225865
Vm50	HY.5B	*Hyalesthes obsoletus*	Italy	KM225864
Vm50	HY.12B	*Hyalesthes obsoletus*	Italy	KM225863
Vm50	Ca13_RF	*Convolvulus arvensis*	Austria	KJ469732
Vm51	C6	*Vitis vinifera*	Italy	HM008618
Vm51	B4	*Vitis vinifera*	Italy	HM008617
Vm51	RA6_I_C	*Convolvulus arvensis*	Italy	JQ977737
Vm52	I_Norheim_C	*Convolvulus arvensis*	Germany	JQ977739
Vm52	Charente-2	*Hyalesthes obsoletus*	France	AM992099
Vm53	P7	*Catharanthus roseus*	Lebanon	AM992100
Vm53	Tsol89	*Vitis vinifera*	Georgia	KT184878
Vm53	Kiqu94	*Vitis vinifera*	Georgia	KT184878
Vm54	P42/11	*Vitis vinifera*	Italy	KJ145356
Vm55	T2_92	*Solanum lycopersicum*	Italy	AM992106
Vm56	36861_SLO_C	*Convolvulus arvensis*	Slovenia	JQ977741
Vm57	CrHo12_721	*Hyalesthes obsoletus*	Austria	KJ469731
Vm58	Mag1	*Vitis vinifera*	Italy	HM008613
Vm58	HY.7N	*Hyalesthes obsoletus*	Italy	KM225868
Vm58	HY.18N	*Hyalesthes obsoletus*	Italy	KM225867
Vm59	MK94	*Vitis vinifera*	Macedonia	KF957606
Vm60	CrHo12_650	*Hyalesthes obsoletus*	Austria	KJ469725
Vm61	Vv12_274	*Vitis vinifera*	Austria	KJ469726
Vm62	425/11	*Vitis vinifera*	Italy	KJ145348
Vm63	Vv12_Ill6	*Vitis vinifera*	Austria	KJ469733
Vm64	Carv1	*Convolvulus arvensis*	Georgia	KT184867
Vm65	Carv2	*Convolvulus arvensis*	Georgia	KT184868
Vm66	Char7	*Vitis vinifera*	Georgia	KT184869
Vm67	Char8	*Vitis vinifera*	Georgia	KT184870
Vm68	Sape19	*Vitis vinifera*	Georgia	KT184871
Vm69	GoMt25	*Vitis vinifera*	Georgia	KT184872
Vm70	Kisi38	*Vitis vinifera*	Georgia	KT184873

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Table S1. Sequence variants of the gene *vmp1* among '*Ca*. P. solani' strains available in GenBank (part IV)

Sequence variant	Strain	Host	Location	Acc. No. *vmp1*
Vm71	Rkat47	*Vitis vinifera*	Georgia	KT184874
Vm71	Sape51	*Vitis vinifera*	Georgia	KT184874
Vm71	Sape62	*Vitis vinifera*	Georgia	KT184874
Vm72	Khik70	*Vitis vinifera*	Georgia	KT184875
Vm73	Amla77	*Vitis vinifera*	Georgia	KT184876
Vm74	Sabu84	*Vitis vinifera*	Georgia	KT184877
Vm75	LN-b	*Salvia miltiorrhiza*	China	KU600116
Vm75	LN-a	*Salvia miltiorrhiza*	China	KU600115
Vm75	LY-6	*Salvia miltiorrhiza*	China	KU600114
Vm75	LY-5	*Salvia miltiorrhiza*	China	KU600113
Vm75	LY-4	*Salvia miltiorrhiza*	China	KU600112
Vm75	SZ-9	*Salvia miltiorrhiza*	China	KU600111
Vm75	SZ-8	*Salvia miltiorrhiza*	China	KU600110
Vm75	SZ-7	*Salvia miltiorrhiza*	China	KU600109
Vm75	LN-3	*Salvia miltiorrhiza*	China	KU600108
Vm75	LN-2	*Salvia miltiorrhiza*	China	KU600107
Vm75	LN-1	*Salvia miltiorrhiza*	China	KU600106
Vm76	G24-13	*Vitis vinifera*	Bosnia & Herzegovina	KT766176
Vm76	5-09	*Vitis vinifera*	Bosnia & Herzegovina	KT766163
Vm76	PM2	*Solanum tuberosum*	Montenegro	KU588193
Vm77	12-11	*Vitis vinifera*	Bosnia & Herzegovina	KT766169
Vm78	HY.8B	*Hyalesthes obsoletus*	Italy	KM225878
Vm79	HY.6B	*Hyalesthes obsoletus*	Italy	KM225873
Vm79	HY.25B	*Hyalesthes obsoletus*	Italy	KM225872
Vm80	Bi.2	*Convolvulus arvensis*	Italy	KM225860
Vm80	HY.53B	*Hyalesthes obsoletus*	Italy	KM225859
	20-09*	*Vitis vinifera*	Bosnia & Herzegovina	KT766164
	G21-13*	*Vitis vinifera*	Bosnia & Herzegovina	KP739861
	154-10*	*Vitis vinifera*	Bosnia & Herzegovina	KT766165
	G4-13*	*Vitis vinifera*	Bosnia & Herzegovina	KT766172
	11-11*	*Vitis vinifera*	Bosnia & Herzegovina	KT766168
	30-09*	*Vitis vinifera*	Bosnia & Herzegovina	KP739860
	3-11*	*Vitis vinifera*	Bosnia & Herzegovina	KT766167
	G22-13*	*Vitis vinifera*	Bosnia & Herzegovina	KT766174
	G6-13*	*Vitis vinifera*	Bosnia & Herzegovina	KT766173
	G23-13*	*Vitis vinifera*	Bosnia & Herzegovina	KT766175
	66-11*	*Vitis vinifera*	Bosnia & Herzegovina	KT766171
	55-11*	*Vitis vinifera*	Bosnia & Herzegovina	KT766170
	155-10*	*Vitis vinifera*	Bosnia & Herzegovina	KT766166
	14-11*	*Vitis vinifera*	Bosnia & Herzegovina	KP739857
	MB11*	*Zea mays*	Bosnia & Herzegovina	KU340854
	MB4*	*Zea mays*	Bosnia & Herzegovina	KU340852
	MB8*	*Zea mays*	Bosnia & Herzegovina	KU340853
	C2_Rgg50*	*Apium graveolens*	Bosnia & Herzegovina	KU340851
	C1_Rgg35/Rqg31*	*Apium graveolens*	Bosnia & Herzegovina	KU340850
	P7*	*Capsicum annuum*	Bosnia & Herzegovina	KU348048
	P6*	*Capsicum annuum*	Bosnia & Herzegovina	KU348047
	P5*	*Capsicum annuum*	Bosnia & Herzegovina	KU348046
	P19*	*Capsicum annuum*	Bosnia & Herzegovina	KU348049

*attribution to sequence variant not possible for the short size of the *vmp1* gene sequence

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Table S2. Nucleotide insertions in *vmp1* sequence variants identified among BNp strains in *Chianti Classico* area

Insertion (nt)	*vmp1* sequence variant[a]											
	vmFi1	**vmFi2**	**vmFi3**	**vmFi4**	**vmFi5**	**vmFi6**	**vmFi7**	**vmFi8**	**vmFi9**	**vmFi10**	**vmFi11**	**vmFi12**
616-619	-	+	+	+	+	+	+	+	+	-	-	+
830-835	-	+	+	+	-	+	+	+	-	-	-	+
865-867	-	+	+	+	+	+	+	+	+	-	-	+
1073-1078	-	-	-	+	-	-	+	-	-	-	-	-
1073-1321	-	+	-	-	-	-	-	+	-	-	-	+
Total size	1290	1551	1302	1308	1296	1302	1308	1551	1296	1290	1290	1551

[a] - : absence of the insertion; + : presence of the insertion

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Table S3. Nucleotide (in white) and amino acid (in grey) sequence identity matrix of *vmp1* sequence variants identified in *Chianti Classico* area

Variant	vmFi1	vmFi2	vmFi3	vmFi4	vmFi5	vmFi6	vmFi7	vmFi8	vmFi9	vmFi10	vmFi11	vmFi12
vmFi1[a]	ID	0,731	0,87	0,866	0,819	0,87	0,864	0,733	0,819	0,997	0,997	0,729
vmFi2[b]	0,789	ID	0,837	0,839	0,723	0,839	0,839	0,998	0,725	0,731	0,731	0,996
vmFi3[c]	0,94	0,838	ID	0,995	0,859	0,997	0,99	0,835	0,861	0,87	0,87	0,835
vmFi4[c]	0,936	0,841	0,995	ID	0,855	0,993	0,995	0,837	0,857	0,866	0,866	0,837
vmFi5[d]	0,913	0,782	0,93	0,926	ID	0,861	0,855	0,721	0,997	0,817	0,817	0,721
vmFi6[e]	0,94	0,839	0,998	0,993	0,932	ID	0,993	0,837	0,864	0,87	0,87	0,837
vmFi7[e]	0,935	0,842	0,993	0,997	0,927	0,994	ID	0,837	0,857	0,864	0,864	0,837
vmFi8[b]	0,79	0,999	0,837	0,84	0,782	0,838	0,841	ID	0,723	0,733	0,733	0,994
vmFi9[d]	0,912	0,783	0,931	0,927	0,999	0,933	0,928	0,782	ID	0,817	0,817	0,723
vmFi10[a]	0,998	0,789	0,94	0,935	0,912	0,94	0,935	0,789	0,911	ID	1	0,729
vmFi11[a]	0,999	0,789	0,94	0,936	0,912	0,94	0,935	0,79	0,912	0,999	ID	0,729
vmFi12[b]	0,789	0,998	0,837	0,84	0,782	0,838	0,841	0,998	0,783	0,789	0,789	ID

[a] New variants vmFi10 and vmFi11 are distinct from the closest related vmFi1 (Vm43) by 2 and 1 SNPs, respectively

[b] New variants vmFi8 and vmFi12 are distinct from the closest related vmFi2 (Vm45) by 1 and 2 SNPs, respectively

[c] New variant vmFi4 is distinct from the closest related vmFi3 (Vm42) by an insertion of 6 nt

[d] New variant vmFi9 is distinct from the closest related vmFi5 (Vm39) by 1 SNP

[e] New variant vmFi7 is distinct from the closest related vmFi6 (Vm41) by an insertion of 6 nt and 1 SNP

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Table S4. Sequence variants of the gene *stamp* among '*Ca*. P. solani' strains available in GenBank (part I)

Sequence variant	Strain	Host	Location	Acc. N. *stamp*
St1	Rqg50	*Reptalus quinquecostatus*	Serbia	KC703019
St1	11-11	*Vitis vinifera*	Bosnia & Herzegovina	KP739852
St1	115/11	*Vitis vinifera*	Italy	KJ145337
St1	17-11	*Vitis vinifera*	Bosnia & Herzegovina	KP739854
St1	20MN	*Vitis vinifera*	Montenegro	KJ926068
St1	353/11	*Vitis vinifera*	Italy	KJ145338
St1	45MN	*Convolvulus arvensis*	Montenegro	KJ926069
St1	72MN	*Vitex agnus-castus*	Montenegro	KJ926070
St1	Ag4a	*Vitis vinifera*	Italy	KJ145377
St1	B1	*Vitis vinifera*	Italy	KJ145378
St1	C45	*Convolvulus arvensis*	Macedonia	KP337319
St1	CrAr12_722_2	*Anaceratagallia ribauti*	Austria	KJ469722
St1	CrHo12_721	*Hyalesthes obsoletus*	Austria	KJ469722
St1	G21-13	*Vitis vinifera*	Bosnia & Herzegovina	KP739856
St1	G22-13	*Vitis vinifera*	Bosnia & Herzegovina	KP739849
St1	G23-13	*Vitis vinifera*	Bosnia & Herzegovina	KP739846
St1	G24-13	*Vitis vinifera*	Bosnia & Herzegovina	KP739847
St1	G4-13	*Vitis vinifera*	Bosnia & Herzegovina	KP739853
St1	G6-13	*Vitis vinifera*	Bosnia & Herzegovina	KP739848
St1	Gb1	*Phaseulus vulgaris*	Serbia	KM977907
St1	Ho375	*Hyalesthes obsoletus*	Montenegro	KJ926071
St1	Ho66-2	*Hyalesthes obsoletus*	Montenegro	KJ926072
St1	HoC202	*Hyalesthes obsoletus*	Macedonia	KP337320
St1	Mp46	*Vitis vinifera*	Italy	KJ145379
St1	P25/11	*Vitis vinifera*	Italy	KJ145339
St1	PM1	*Solanum tuberosum*	Montenegro	KU588188
St1	PM2	*Solanum tuberosum*	Montenegro	KU588189
St1	PS8	*Solanum tuberosum*	Serbia	KP877599
St1	PS8Ho	*Hyalesthes obsoletus*	Serbia	KP877600
St1	PS8Rp	*Reptalus panzeri*	Serbia	KP877601
St1	PS9	*Solanum tuberosum*	Serbia	KP877602
St1	Rpg47	*Reptalus panzeri*	Serbia	KC703020
St1	Vv12_Ill6	*Vitis vinifera*	Austria	KJ469722
St1	Vv5	*Vitis vinifera*	Serbia	KC703021
St2	Rqg31	*Reptalus quinquecostatus*	Serbia	KC703017
St2	Br8	*Convolvulus arvensis*	Croatia	KJ573597
St2	C2_Rgg50	*Apium graveolens*	Bosnia & Herzegovina	KU295506
St2	Ho41-2	*Hyalesthes obsoletus*	Montenegro	KJ926065
St2	P10	*Capsicum annuum*	Bosnia & Herzegovina	KU295504
St2	P6	*Capsicum annuum*	Bosnia & Herzegovina	KU295502
St2	PS4	*Solanum tuberosum*	Serbia	KP877588
St2	PS4Ho	*Hyalesthes obsoletus*	Serbia	KP877589
St2	PS5	*Solanum tuberosum*	Serbia	KP877590
St2	PS5Ho	*Hyalesthes obsoletus*	Serbia	KP877591
St2	PS5Rp	*Reptalus panzeri*	Serbia	KP877592
St2	PS6	*Solanum tuberosum*	Serbia	KP877593
St2	PS6Ho	*Hyalesthes obsoletus*	Serbia	KP877594
St2	PS6Rq	*Reptalus quinquecostatus*	Serbia	KP877595
St2	Vv12_Kn6	*Vitis vinifera*	Austria	KJ469724
St2	Vv17	*Vitis vinifera*	Serbia	KC703018

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Table S4. Sequence variants of the gene *stamp* among '*Ca*. P. solani' strains available in GenBank (part II)

Sequence variant	Strain	Host	Location	Acc. N. *stamp*
St3	16MN	*Vitis vinifera*	Montenegro	KJ926073
St3	30-09	*Vitis vinifera*	Bosnia & Herzegovina	KP739851
St3	43MN	*Convolvulus arvensis*	Montenegro	KJ926074
St3	79MN	*Vitex agnus-castus*	Montenegro	KJ926075
St3	Ho389	*Hyalesthes obsoletus*	Montenegro	KJ926076
St3	MK66	*Vitis vinifera*	Macedonia	KF957608
St3	P5	*Capsicum annuum*	Bosnia & Herzegovina	KU295501
St3	P7	*Catharanthus roseus*	Lebanon	FN813258
St3	PS7	*Solanum tuberosum*	Serbia	KP877596
St3	PS7Ho	*Hyalesthes obsoletus*	Serbia	KP877597
St3	PS7Rp	*Reptalus panzeri*	Serbia	KP877598
St3	Rpm35	*Reptalus panzeri*	Serbia	KC703015
St3	Vv12_751	*Vitis vinifera*	Austria	KJ469723
St4	G2	*Vitis vinifera*	Macedonia	KP337318
St4	GR328	*Capsicum annuum*	Greece	FN813253
St4	Ho10-2	*Hyalesthes obsoletus*	Montenegro	KJ926067
St4	MB11	*Zea mays*	Bosnia & Herzegovina	KU295509
St4	MB4	*Zea mays*	Bosnia & Herzegovina	KU295507
St4	MB6	*Zea mays*	Bosnia & Herzegovina	KU295508
St4	PS1	*Solanum tuberosum*	Serbia	KP877583
St4	PS1Rp	*Reptalus panzeri*	Serbia	KP877584
St4	PS1Rq	*Reptalus quinquecostatus*	Serbia	KP877585
St4	Rpg39	*Reptalus panzeri*	Serbia	KC703009
St4	Rpm34	*Reptalus panzeri*	Serbia	KC703010
St4	Rqg60	*Reptalus quinquecostatus*	Serbia	KC703011
St4	STOL	*Capsicum annuum*	Serbia	FN813261
St4	Vexp Rpg11	*Reptalus panzeri*	Serbia	KC703013
St4	Vexp Rpm5	*Reptalus panzeri*	Serbia	KC703014
St4	Vv21	*Vitis vinifera*	Serbia	KC703012
St5	215/11	*Vitis vinifera*	Italy	KJ145329
St5	287/11	*Vitis vinifera*	Italy	KJ145332
St5	315/11	*Vitis vinifera*	Italy	KJ145330
St5	425/11	*Vitis vinifera*	Italy	KJ145335
St5	78/11	*Vitis vinifera*	Italy	KJ145334
St5	Ca13_RF	*Convolvulus arvensis*	Austria	KJ469721
St5	CrHo12_601	*Hyalesthes obsoletus*	Austria	KJ469721
St5	GGY	*Vitis vinifera*	Germany	FN813256
St5	HoC205	*Hyalesthes obsoletus*	Macedonia	KP337315
St5	LA6_I_C	*Convolvulus arvensis*	Germany	JQ977720
St5	NGA9	*Hyalesthes obsoletus*	Slovenia	FN813262
St5	P136/11	*Vitis vinifera*	Italy	KJ145336
St5	P51/11	*Vitis vinifera*	Italy	KJ145331
St5	P75/11	*Vitis vinifera*	Italy	KJ145333
St5	Vv12_752	*Vitis vinifera*	Austria	KJ469721
St5	Vv12_754	*Vitis vinifera*	Austria	KJ469721
St6	MK44	*Vitis vinifera*	Macedonia	KF957607
St6	S7	*Urtica dioica*	Slovenia	JQ977719
St7	S6	*Urtica dioica*	Italy	JQ977718

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Table S4. Sequence variants of the gene *stamp* among '*Ca*. P. solani' strains available in GenBank (part III)

Sequence variant	Strain	Host	Location	Acc. N. *stamp*
St8	49MN	*Urtica dioica*	Montenegro	KJ926078
St8	4MN	*Vitis vinifera*	Montenegro	KJ926077
St8	BN-Yan1	*Vitis vinifera*	Italy	KX151182
St8	Ho13_838	*Hyalesthes obsoletus*	Austria	KJ469720
St8	Ho13-8	*Hyalesthes obsoletus*	Montenegro	KJ926079
St8	HoU190	*Hyalesthes obsoletus*	Macedonia	KP337321
St8	S5	*Urtica dioica*	Italy	JQ977717
St8	SB5	*Vitis vinifera*	Croatia	FN813266
St9	60/11	*Vitis vinifera*	Italy	KJ145345
St9	7-9	*Vitis vinifera*	Croatia	KP274915
St9	Aa25	*Vitis vinifera*	Italy	KJ145387
St9	Aaq29	*Vitis vinifera*	Italy	KJ145388
St9	Ho13_1006	*Hyalesthes obsoletus*	Austria	KJ469718
St9	Mcil	*Vitis vinifera*	Italy	KJ145385
St9	S2	*Urtica dioica*	Slovenia	JQ977714
St10	LG	*Solanum lycopersicum*	France	FN813259
St10	PO	*Hyalesthes obsoletus*	France	FN813270
St11	19-25	*Vitis vinifera*	Germany	FN813267
St11	33MN	*Vitis vinifera*	Montenegro	KJ926080
St11	67MN	*Urtica dioica*	Montenegro	KJ926081
St11	CrHo12_650	*Hyalesthes obsoletus*	Austria	KJ469716
St11	E	*Hyalesthes obsoletus*	Germany	FN813263
St11	G1	*Vitis vinifera*	Macedonia	KP337322
St11	GBr2	*Vitis vinifera*	Croatia	KJ573590
St11	GBr4	*Vitis vinifera*	Croatia	KJ573591
St11	GVu1	*Vitis vinifera*	Croatia	KJ573592
St11	GVu2	*Vitis vinifera*	Croatia	KJ573593
St11	H17	*Hyalesthes obsoletus*	Croatia	KJ573594
St11	H18	*Hyalesthes obsoletus*	Croatia	KJ573595
St11	H21	*Hyalesthes obsoletus*	Croatia	KJ573596
St11	Ho36-8	*Hyalesthes obsoletus*	Montenegro	KJ926082
St11	HoU17	*Hyalesthes obsoletus*	Macedonia	KP337323
St11	MK94	*Vitis vinifera*	Macedonia	KF957609
St12	L646	*Lavandula angustifolia*	France	FN813265
St13	GR13	*Vitis vinifera*	Greece	FN813264
St14	C	*Solanum lycopersicum*	France	FN813260
St15	P7	*Capsicum annuum*	Bosnia & Herzegovina	KU295503
St15	Tsol89	*Vitis vinifera*	Georgia	KT184885
St15	Kiqu84	*Vitis vinifera*	Georgia	KT184885
St16	H299	*Hyalesthes obsoletus*	France	FN813254
St16	L973	*Lavandula angustifolia*	France	FN813255
St17	Ate17	*Vitis vinifera*	Italy	KJ145386
St18	266/11	*Vitis vinifera*	Italy	KJ145344
St18	Aaq1	*Vitis vinifera*	Italy	KJ145383
St18	Mdxsain	*Vitis vinifera*	Italy	KJ145384
St19	CrHo13_1183	*Hyalesthes obsoletus*	Austria	KJ469719
St19	S3	*Urtica dioica*	Slovenia	JQ977715
St20	136/11	*Vitis vinifera*	Italy	KJ145340
St20	166/11	*Vitis vinifera*	Italy	KJ145343
St20	Ate7	*Vitis vinifera*	Italy	KJ145381
St20	Mca21	*Vitis vinifera*	Italy	KJ145382
St20	P10/11	*Vitis vinifera*	Italy	KJ145342
St20	P42/11	*Vitis vinifera*	Italy	KJ145341

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Table S4. Sequence variants of the gene *stamp* among '*Ca*. P. solani' strains available in GenBank (part IV)

Sequence variant	Strain	Host	Location	Acc. N. *stamp*
St21	Aa16	*Vitis vinifera*	Italy	KJ145380
St22	Mvercer2	*Vitis vinifera*	Italy	KJ145375
St23	Lot et Garonne	*Solanum lycopersicum*	France	FN813257
St24	HoU93	*Hyalesthes obsoletus*	Macedonia	KP337314
St24	U79	*Hyalesthes obsoletus*	Macedonia	KP337313
St25	HoU80	*Hyalesthes obsoletus*	Macedonia	KP337309
St26	G5	*Hyalesthes obsoletus*	Macedonia	KP337310
St26	HoU85	*Hyalesthes obsoletus*	Macedonia	KP337311
St27	U70	*Urtica dioica*	Macedonia	KP337312
St28	HoC68	*Hyalesthes obsoletus*	Macedonia	KP337316
St28	PS3	*Solanum tuberosum*	Serbia	KP877587
St29	Vv12_274	*Vitis vinifera*	Austria	KJ469717
St30	10MN	*Vitis vinifera*	Montenegro	KJ926066
St30	4-9	*Vitis vinifera*	Croatia	KP274914
St30	G25	*Vitis vinifera*	Macedonia	KP337317
St30	PS10Ho	*Hyalesthes obsoletus*	Serbia	KP877603
St30	PS10Rq	*Reptalus quinquecostatus*	Serbia	KP877604
St30	Vv24	*Vitis vinifera*	Serbia	KC703022
St31	BG4560	*Vitis vinifera*	Bulgaria	FN813252
St31	PS2	*Solanum tuberosum*	Serbia	KP877586
St31	Rqg42	*Reptalus quinquecostatus*	Serbia	KC703016
St32	Mp49	*Vitis vinifera*	Italy	KJ145376
St33	OSESLO2	*Hyalesthes obsoletus*	Slovenia	FN813269
St33	Rome15	*Hyalesthes obsoletus*	Italy	FN813268
St33	S4	*Urtica dioica*	Italy	JQ977716
St34	S1	*Urtica dioica*	Germany	JQ977713
St35	Carv1	*Convolvulus arvensis*	Georgia	KT184879
St36	Carv2	*Convolvulus arvensis*	Georgia	KT184880
St37	Char7	*Convolvulus arvensis*	Georgia	KT184881
St37	Kisi38	*Vitis vinifera*	Georgia	KT184881
St37	Rkat47	*Vitis vinifera*	Georgia	KT184881
St37	Sape51	*Vitis vinifera*	Georgia	KT184881
St37	Sape62	*Vitis vinifera*	Georgia	KT184881
St38	Char8	*Convolvulus arvensis*	Georgia	KT184882
St38	Sape19	*Vitis vinifera*	Georgia	KT184882
St38	GoMt25	*Vitis vinifera*	Georgia	KT184882
St39	Amla77	*Vitis vinifera*	Georgia	KT184883
St40	Sabu84	*Vitis vinifera*	Georgia	KT184884
St41	20-09	*Vitis vinifera*	Bosnia & Herzegovina	KT766177
St42	154-10	*Vitis vinifera*	Bosnia & Herzegovina	KP739855
St43	3-11	*Vitis vinifera*	Bosnia & Herzegovina	KP739850
St44	C1_Rgg35/Rqg31	*Apium graveolens*	Bosnia & Herzegovina	KU295505
St45	Ho1152	*Hyalesthes obsoletus*	Montenegro	KM977906
St46	RQ161	*Reptalus quinquecostatus*	France	LN823951

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Table S5. Nucleotide (in white) and amino acid (in grey) sequence identity matrix of *stamp* sequence variants identified in *Chianti Classico* area

Variant	stFi1	stFi2	stFi3	stFi4	stFi5	stFi6	stFi7	stFi8	stFi9	stFi10	stFi11	stFi12	stFi13	stFi14	stFi15	stFi16
stFi1[a]	ID	0,936	0,93	0,929	0,923	0,898	0,936	0,929	0,891	0,892	0,923	1	1	0,993	0,923	0,981
stFi2[b]	0,972	ID	0,924	0,993	0,859	0,859	0,911	0,917	0,853	0,886	0,987	0,936	0,936	0,929	0,974	0,943
stFi3[c]	0,97	0,964	ID	0,917	0,86	0,886	0,936	0,93	0,854	0,962	0,911	0,93	0,93	0,924	0,911	0,93
stFi4	0,97	0,997	0,962	ID	0,853	0,853	0,905	0,91	0,847	0,879	0,98	0,929	0,929	0,923	0,98	0,936
stFi5[a]	0,968	0,94	0,939	0,938	ID	0,878	0,879	0,872	0,917	0,86	0,847	0,923	0,923	0,923	0,859	0,905
stFi6[d]	0,959	0,94	0,947	0,938	0,949	ID	0,892	0,929	0,878	0,867	0,859	0,898	0,898	0,904	0,866	0,892
stFi7[a]	0,972	0,958	0,972	0,955	0,949	0,951	ID	0,943	0,854	0,911	0,911	0,936	0,936	0,93	0,911	0,93
stFi8[e]	0,972	0,964	0,966	0,962	0,949	0,976	0,97	ID	0,84	0,917	0,917	0,929	0,929	0,929	0,929	0,93
stFi9[a]	0,959	0,943	0,937	0,94	0,966	0,945	0,943	0,936	ID	0,854	0,853	0,891	0,891	0,885	0,84	0,873
stFi10[c]	0,955	0,949	0,985	0,947	0,937	0,941	0,962	0,96	0,935	ID	0,898	0,892	0,892	0,892	0,898	0,892
stFi11[b]	0,968	0,995	0,96	0,993	0,936	0,94	0,958	0,964	0,943	0,953	ID	0,923	0,923	0,917	0,974	0,93
stFi12[a]	0,997	0,97	0,968	0,968	0,97	0,957	0,97	0,97	0,957	0,953	0,966	ID	1	0,993	0,923	0,981
stFi13[a]	0,997	0,97	0,968	0,968	0,97	0,962	0,97	0,974	0,957	0,953	0,966	0,995	ID	0,993	0,923	0,981
stFi14[a]	0,995	0,968	0,966	0,966	0,968	0,964	0,968	0,972	0,955	0,955	0,964	0,993	0,993	ID	0,923	0,974
stFi15[b]	0,964	0,987	0,955	0,989	0,94	0,945	0,953	0,968	0,934	0,953	0,987	0,962	0,966	0,964	ID	0,93
stFi16[a]	0,987	0,972	0,974	0,97	0,955	0,951	0,972	0,968	0,947	0,96	0,968	0,985	0,985	0,983	0,964	ID

[a] New variants distinct from the closest related stFi1 (St10) by 15 (StFi5), 9 plus insertion (StFi7), 20 (stFi9), 1 (stFi12), 1 (stFi13), 2 (stFi14) and 3 plus insertion (stFi16) SNP(s)

[b] New variants distinct from the closest related stFi2 (St5) by 2 (StFi11) and 6 (stFi15) SNPs

[c] New variant stFi10 distinct from the closest related stFi3 (St18) by 7 SNPs

[d] New variant stFi6 distinct from the closest related St36 (not found in the study vineyard) by 7 SNPs

[e] New variant stFi8 distinct from the closest related St15 and St37 (not found in the study vineyard) by 7 SNPs

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Table S6. *vmp1*/*stamp* types of '*Ca.* P. solani' strains available in GenBank (part I)

vmp1	*stamp*	Strain	Host	Country
Vm10	St2	Rqg31	*Reptalus quinquecostatus*	Serbia
Vm10	St2	**Vv17**	*Vitis vinifera*	Serbia
Vm10	St4	Rpg39	*Reptalus panzeri*	Serbia
Vm10	St4	Rpm34	*Reptalus panzeri*	Serbia
Vm10	St4	Rqg60	*Reptalus quinquecostatus*	Serbia
Vm10	St4	**STOL**	*Capsicum annuum*	Serbia
Vm10	St4	Vexp Rpg11	*Reptalus panzeri*	Serbia
Vm10	St4	Vexp Rpm5	*Reptalus panzeri*	Serbia
Vm10	St4	Vv21	*Vitis vinifera*	Serbia
Vm11	St5	**LA6_I_C**	*Convolvulus arvensis*	Germany
Vm12	St5	**GGY**	*Vitis vinifera*	Germany
Vm14	St2	**Vv12_Kn6**	*Vitis vinifera*	Austria
Vm14	St3	**Vv12_751**	*Vitis vinifera*	Austria
Vm14	St5	**Vv12_754**	*Vitis vinifera*	Austria
Vm15	St9	60/11	*Vitis vinifera*	Italy
Vm15	St9	Aa25	*Vitis vinifera*	Italy
Vm15	St9	**Ho13_1006**	*Hyalesthes obsoletus*	Austria
Vm18	St8	**Ho13_838**	*Hyalesthes obsoletus*	Austria
Vm23	St19	**CrHo13_1183**	*Hyalesthes obsoletus*	Austria
Vm25	St6	**MK44**	*Vitis vinifera*	Macedonia
Vm27	St21	**Aa16**	*Vitis vinifera*	Italy
Vm28	St1	**Rqg50**	*Reptalus quinquecostatus*	Serbia
Vm28	St1	17-11	*Vitis vinifera*	Bosnia & Herzegovina
Vm28	St1	CrAr12_722_2	*Anaceratagallia ribauti*	Austria
Vm28	St1	Rpg47	*Reptalus panzeri*	Serbia
Vm28	St5	**Vv12_752**	*Vitis vinifera*	Austria
Vm28	St18	**Aaq1**	*Vitis vinifera*	Italy
Vm28	St20	136/11	*Vitis vinifera*	Italy
Vm28	St20	166/11	*Vitis vinifera*	Italy
Vm28	St20	**Mca21**	*Vitis vinifera*	Italy
Vm28	St20	P10/11	*Vitis vinifera*	Italy
Vm33	St5	**CrHo12_601**	*Hyalesthes obsoletus*	Austria
Vm34	St11	**19-25**	*Vitis vinifera*	Germany
Vm35	St10	**PO**	*Hyalesthes obsoletus*	France
Vm36	St31	**Rqg42**	*Reptalus quinquecostatus*	Serbia
Vm38	St10	**LG**	*Solanum lycopersicum*	France
Vm39	St5	**78/11**	*Vitis vinifera*	Italy
Vm40	St32	**Mp49**	*Vitis vinifera*	Italy
Vm44	St22	**Mvercer2**	*Vitis vinifera*	Italy
Vm45	St5	**315/11**	*Vitis vinifera*	Italy
Vm45	St5	P136/11	*Vitis vinifera*	Italy
Vm45	St5	P75/11	*Vitis vinifera*	Italy
Vm46	St1	115/11	*Vitis vinifera*	Italy
Vm46	St1	353/11	*Vitis vinifera*	Italy
Vm46	St1	Ag4a	*Vitis vinifera*	Italy
Vm46	St1	**Mp46**	*Vitis vinifera*	Italy
Vm46	St1	PM1	*Solanum tuberosum*	Montenegro
Vm46	St1	Vv5	*Vitis vinifera*	Serbia
Vm46	St3	**Rpm35**	*Reptalus panzeri*	Serbia

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Table S6. *vmp1*/*stamp* types of '*Ca*. P. solani' strains available in GenBank (part II)

vmp1	***stamp***	**Strain**	**Host**	**Country**
Vm46	St5	**287/11**	*Vitis vinifera*	Italy
Vm46	St30	**Vv24**	*Vitis vinifera*	Serbia
Vm50	St5	**Ca13_RF**	*Convolvulus arvensis*	Austria
Vm53	St15	**P7**	*Catharanthus roseus*	Lebanon
Vm53	St15	Tsol89	*Vitis vinifera*	Georgia
Vm53	St15	Kiqu84	*Vitis vinifera*	Georgia
Vm54	St20	**P42/11**	*Vitis vinifera*	Italy
Vm57	St1	**CrHo12_721**	*Hyalesthes obsoletus*	Austria
Vm59	St11	**MK94**	*Vitis vinifera*	Macedonia
Vm60	St11	**CrHo12_650**	*Hyalesthes obsoletus*	Austria
Vm61	St29	**Vv12_274**	*Vitis vinifera*	Austria
Vm62	St5	**425/11**	*Vitis vinifera*	Italy
Vm63	St1	**Vv12_Ill6**	*Vitis vinifera*	Austria
Vm64	St35	**Carv1**	*Convolvulus arvensis*	Georgia
Vm65	St36	**Carv2**	*Convolvulus arvensis*	Georgia
Vm66	St37	**Char7**	*Convolvulus arvensis*	Georgia
Vm67	St38	**Char8**	*Convolvulus arvensis*	Georgia
Vm68	St38	**Sape19**	*Vitis vinifera*	Georgia
Vm69	St38	**GoMt25**	*Vitis vinifera*	Georgia
Vm70	St37	**Kisi38**	*Vitis vinifera*	Georgia
Vm71	St37	**Rkat47**	*Vitis vinifera*	Georgia
Vm71	St37	Sape51	*Vitis vinifera*	Georgia
Vm71	St37	Sape62	*Vitis vinifera*	Georgia
Vm73	St39	**Amla77**	*Vitis vinifera*	Georgia
Vm74	St40	**Sabu84**	*Vitis vinifera*	Georgia
Vm76	St1	**G24-13**	*Vitis vinifera*	Bosnia & Herzegovina
Vm76	St1	PM2	*Solanum tuberosum*	Montenegro

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