



Prevalence of a '*Candidatus Phytoplasma solani*' strain, so far associated only with other hosts, in Bois noir-affected grapevines within Tuscan vineyards

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Key Words:	grapevine yellows, stolbur, multi-locus sequence analysis, <i>Vitis vinifera</i> , phylogenetic analysis

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3 1 **Prevalence of a '*Candidatus Phytoplasma solani*' strain, so far**
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6 2 **associated only with other hosts, in Bois noir-affected grapevines**
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9 3 **within Tuscan vineyards**
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48 21 **Running title:** Prevalence of unusual '*Ca. P. solani*' strain in Tuscan grapevines
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26 SUMMARY

27 Due to its complex epidemiological cycle, including several polyphagous insect vectors and
28 host plants, and the absence of efficient control strategies, Bois noir (BN) disease of grapevine is
29 encroaching wider territories in the main viticultural areas worldwide. Molecular approaches
30 allowed to increase the knowledge about its etiological agent (Bois noir phytoplasma, BNp;
31 '*Candidatus* Phytoplasma solani' species), revealing interesting features concerning BNp
32 population structure and dynamics and transmission routes in vineyard agro-ecosystems. In the
33 present study, a multi-locus sequence typing approach (*vmp1* and *stamp* genes) was utilized for
34 describing the genetic diversity among BNp strain populations in 17 vineyards localized in two
35 distinct geographic areas in Tuscany (central Italy). The results confirmed that BNp ecology in
36 Tuscan vineyards is mainly associated to the bindweed-related host system, and allowed the
37 identification of 14 BNp *vmp1/stamp* genotypes. Interestingly, the prevalent genotype (Vm43/St10)
38 was never found in grapevines outside of Tuscany. Moreover, statistical analyses showed
39 significant differences between the composition of BNp strain populations identified in grapevines
40 from north-western and central-eastern Tuscany. These results reinforce the hypothesis that distinct
41 geographic areas, probably associated with different ecological niches, can drive the selection of
42 BNp strains, also favouring the entrance of unusual '*Ca. Phytoplasma solani*' genotypes in
43 vineyards.

44
45 **Keywords:** grapevine yellows; stolbur; multi-locus sequence analysis; *Vitis vinifera*; phylogenetic
46 analysis

52 INTRODUCTION

53 Bois Noir (BN), one of the most important diseases of the grapevine yellows (GY) phytoplasma-
54 associated complex, causes severe crop losses in vine growing areas in Euro-Mediterranean
55 countries (Belli *et al.*, 2010), and in restricted zones of South America (Chile) (Gajardo *et al.*, 2009)
56 and Asia (China, Middle East) (Choueiri *et al.*, 2002; Duduk *et al.*, 2010; Salem *et al.*, 2013;
57 Mirchenari *et al.*, 2015). In almost all varieties of *Vitis vinifera* L. BN induces typical GY
58 symptoms, including desiccation of inflorescences, berry shrivel, leaf discolorations, reduction of
59 growth and irregular ripening of wood (Belli *et al.*, 2010). On the basis of unique biological
60 properties and exclusive molecular markers within multiple genes (*16S rRNA*, *tufB*, *rplV-rpsC*,
61 *secY*), the etiological agent of BN (BN phytoplasma, BNp) has been attributed to the species
62 ‘*Candidatus Phytoplasma solani*’ (subgroup 16SrXII-A) (Quaglino *et al.*, 2013).

63 In Europe and Mediterranean basin, ‘*Ca. P. solani*’ strains are transmitted from plant-to-plant
64 mainly by *Hyalesthes obsoletus* Signoret (Homoptera: Cixiidae), a polyphagous leafhopper living
65 preferentially on nettle (*Urtica dioica* L.), bindweed (*Convolvulus arvensis* L.), mugwort
66 (*Artemisia vulgaris* L.), and chaste tree (*Vitex agnus-castus* L.) (Alma *et al.*, 1988; Maixner, 1994;
67 Langer & Maixner, 2004; Sharon *et al.*, 2005). Further studies reported the presence of other insect
68 vectors (e.g. *Anaceratagallia ribauti*, *Reptalus panzeri*, and *R. quinquecostatus*) and host plants
69 (Riedle-Bauer *et al.*, 2008; Cvrković *et al.*, 2014; Landi *et al.*, 2015; Marchi *et al.*, 2015; Mori *et*
70 *al.*, 2015; Oliveri *et al.*, 2015; Chuche *et al.*, 2016; Kosovac *et al.*, 2016), indicating that this
71 phytoplasma species exists in varied ecosystems, where selection conceivably alters strain
72 populations. This hypothesis implies that ecological relationships of BNp (‘*Ca. P. solani*’ strains
73 associated with BN), possibly influenced by climatic and geographic features of vineyard agro-
74 ecosystems in different regions, may be reflected in intra-species strain diversity (Quaglino *et al.*,
75 2009, 2017).

76 Sequence analysis of *tufB* gene revealed that three *tuf*-types of BNp were present in diseased
77 grapevines, as well as in specific plant hosts, determining two main natural ecologies of BNp

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3 78 related to nettle [BNp *tuf*-type a and ab (formerly known as b2)] and bindweed (BNp *tuf*-type b)
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5 79 (Langer & Maixner, 2004; Aryan *et al.*, 2014; Atanasova *et al.*, 2015). Multi-locus sequence typing
6
7 80 (MLST), based on molecular characterization of more variable genes, such as *secY*, *vmp1* and
8
9 81 *stamp*, evidenced a large variability among BNp strains within the *tuf*-types (Foissac *et al.*, 2013;
10
11 82 Quaglino *et al.*, 2016). Molecular approaches, using *vmp1*- and *stamp*-based molecular markers of
12
13 83 BNp diversity, allowed to increase the knowledge of the BNp population structure and dynamics
14
15 84 (Murolo & Romanazzi, 2015; Quaglino *et al.*, 2016) and their transmission routes throughout
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17 85 vineyards and their surroundings (Mori *et al.*, 2015; Kosovac *et al.*, 2016). Interestingly, several
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19 86 studies demonstrated that BNp strains associated with nettle (*tuf*-type a and ab) and bindweed (*tuf*-
20
21 87 type b) ecologies grouped in separate phylogenetic clusters determined by nucleotide sequence
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23 88 analysis of the gene *stamp* (Aryan *et al.*, 2014; Atanasova *et al.*, 2015; Plavec *et al.*, 2015).

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25
26 89 In the present study, a multi-locus sequence typing approach, based on the molecular
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28 90 characterization of *vmp1* and *stamp* genes, was utilized for describing the genetic diversity among
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30 91 BNp strain populations in 17 vineyards localized in two distinct geographic areas of Tuscany.

31 32 33 34 35 93 **MATERIALS AND METHODS**

36 37 94 38 39 95 **Field surveys**

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41 96 During a field survey for grapevine yellows (GY), carried out in the late summer of 2016 in
42
43 97 17 vineyards located in north-western (districts of Lucca and Massa) and central-eastern (districts of
44
45 98 Arezzo, Firenze and Siena) Tuscany Region, 75 grapevine (*Vitis vinifera* L.) plants, cv. Chardonnay
46
47 99 (38) and Sangiovese (37), showing GY symptoms were observed and sampled for phytoplasma
48
49 100 detection and characterization (Table 1). For each symptomatic plant, 10-15 leaves were collected
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51 101 and their fresh central midribs were dissected and stored at -20°C until DNA extraction. Leaves
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53 102 collected in the screenhouse of the Department of Agriculture, Food and Environment (DAFE,
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55 103 University of Pisa, Italy) from *V. vinifera* cv. Chardonnay and Sangiovese were used as healthy

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3 104 control plants (HC), while leaves collected by *V. vinifera* plants, previously found infected by ‘*Ca.*
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5 105 *P. solani*’ (subgroup 16SrXII-A) and Flavescence dorée phytoplasmas (FDp) (subgroups 16SrV-C
6
7 106 or -D) were used as infected controls (ICs) (Pierro *et al.*, 2018).
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10 11 12 108 **Phytoplasma detection**

13 109 DNA was extracted with 2% cetyltrimethylammonium bromide (CTAB) based buffer from leaf
14
15 110 veins according to the protocol described by Li *et al.* (2008), with some modifications according to
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18 111 Pierro *et al.* (2018).

19
20 112 Specific detection of phytoplasmas associated with BN (BNp) and FD (FDp), the GY mainly
21
22 113 present in Europe, was carried out by amplification of 16S ribosomal DNA through TaqMan assay
23
24 114 using the Rotor-Gene *Q* (Qiagen, Germany) following reaction conditions as described by Angelini
25
26 115 *et al.* (2007). The template used in the assay was a 1:10 dilution of the DNA extracted from the
27
28 116 samples. The grapevine chloroplast *chaperonin 21* gene and DNA extracted from HC plants and
29
30 117 ICs were used as endogenous, negative and positive controls, respectively. Threshold cycle (Ct) <
31
32 118 37 was associated with the presence of GY phytoplasmas (Angelini *et al.*, 2007).
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36 37 38 120 **BNp characterization by MLST**

39 121 Nucleotide sequence analyses of the non-ribosomal genomic regions *vmp1* and *stamp* were
40
41 122 carried out on BNp strains detected in symptomatic grapevine plants.
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43
44 123 Direct PCR using StolH10F1/StolH10R1 primer pair followed by nested PCR with the
45
46 124 TYPH10F/TYPH10R primer pair, using mixtures and PCR conditions as previously described
47
48 125 (Cimerman *et al.*, 2009; Fialová *et al.*, 2009), were utilized to obtain the amplification of the *vmp1*
49
50 126 gene in an automated thermal cycler C1000 Cyclo Touch (Bio-Rad, USA). The presence of the
51
52 127 nested PCR products was verified through electrophoresis on 1% agarose gels in Tris-borate-EDTA
53
54 128 (TBE) buffer. The *vmp1* nested PCR products were sequenced in both strands (Sanger method, 5X
55
56 129 coverage per base position) by a commercial service (Eurofins Genomics, Germany). Nucleotide
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3 130 sequences were assembled by the Contig Assembling Program and trimmed to the annealing sites of
4
5 131 the nested PCR primer pair in the software BioEdit, version 7.2.6 (Hall, 1999).

6
7 132 Direct PCR using StampF/StampR0 primer pair followed by nested PCR with the StampF1/
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9 133 StampR1 primer pair, using mixtures and PCR conditions as described by Fabre *et al.* (2011) was
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11 134 utilized to obtain the amplification of the *stamp* gene in an automated thermal cycler C1000 Cycler
12
13 135 Touch. The presence of the nested PCR products was verified through electrophoresis, and *stamp*
14
15 136 nested amplicons were sequenced in both strands and assembled as described above for the gene
16
17 137 *vmp1*.

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19
20 138 Nucleotide sequences of the genes *vmp1* and *stamp*, amplified from the BNp strains detected
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22 139 in the examined vineyards, were aligned using the software BioEdit in ClustalW Multiple
23
24 140 Alignment program and analysed by Sequence Identity Matrix to estimate their genetic diversity.
25
26 141 *Vmp1* and *stamp* sequence variants, identified in the present study, were aligned and compared with
27
28 142 representative sequences of previously defined sequence variants (updated from Pierro *et al.*, 2018)
29
30 143 (Table S1 and S2); a nucleotide sequence identity of 100% was necessary for the attribution to such
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32 144 sequence variants. Based on *vmp1* and *stamp* sequence variant, each BNp strain was attributed to
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34 145 collective *vmp1/stamp* genotype.
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38 39 147 **BNp phylogenetic analysis**

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41 148 *Vmp1* and *stamp* gene nucleotide sequences of BNp representative strains of Vm (*vmp1*) and St
42
43 149 (*stamp*) sequence variants, identified in this (Table 2 and 3) and in previous studies (Table S1 and
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45 150 S2), were aligned and used for generating unrooted phylogenetic trees by minimum evolution
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47 151 method carried out using the Jukes-Cantor model and bootstrap replicated 1000 times in the
48
49 152 MEGA6 software (Tamura *et al.*, 2013). Moreover, representative nucleotide sequences of *vmp1*
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51 153 and *stamp* genes were concatenated by BioEdit and employed for phylogenetic analyses. All
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53 154 positions with less than 95.0% site coverage were eliminated. That is, fewer than 5% alignment
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55 155 gaps, missing data, and ambiguous bases were allowed at any position.
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157 **Statistical analyses**

158 Statistical analyses were carried out to estimate possible statistically significant differences in
159 the distribution of BNp strains grouped in distinct *vmp1/stamp* phylogenetic clusters, identified in
160 the present study, in north-western and central-eastern Tuscany, the two distinct geographic areas
161 considered in the present study. In detail, chi square test (χ^2 test) was performed in SPSS statistical
162 package for Windows, v. 24.0 (IBM Corporation, Armonk, NY).

163

164 **RESULTS**

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166 **Bois Noir phytoplasma detection**

167 TaqMan assay, performed using specific primer pairs for the amplification of BNp 16S rDNA
168 in Real-time PCR, detected BNp in all the 75 symptomatic grapevine plants analysed, while FDP
169 was not found. Assay reliability was validated by the amplification signals of the ICs ($30 < Ct < 32$ for
170 both BNp and FDP), while HC and reaction mixtures gave no amplification signals.

171

172 **Multiple gene sequence typing of BNp strains**

173 Nested-PCR reactions allowed the amplification of *vmp1* and *stamp* gene respectively in 34
174 (45%) and 63 (84%) out of the 75 symptomatic grapevine plants found BNp-infected by Real-time
175 PCR assay; no amplification of the analyzed genes was obtained in nine (12%) out of the 75 BNp-
176 infected grapevines.

177 The 34 *vmp1* nested-PCR fragments of the expected size, amplified using the specific primer
178 pair TYPH10F/R, were sequenced. Based on sequence identities, eight distinct *vmp1* sequence
179 variants (VmTus1 to VmTus8) were detected (Table 1). Comparison with *vmp1* sequence variant
180 updated dataset (Table S1) revealed that sequence variants VmTus1, VmTus2 and VmTus3 shared
181 100% sequence identity with previously described sequence variants Vm39, Vm41 and Vm43,

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2
3 182 respectively. Five *vmp1* sequence variants (VmTus4 to VmTus8) were described for the first time in
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5 183 the present study and named Vm88 to Vm92 (Table 2). In detail, sequence variants VmTus4 and
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7 184 VmTus5 shared the best sequence identity with previously described sequence variant Vm8 (95.2%
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9 185 and 97.3%, respectively), VmTus6 with Vm15 (99%), VmTus7 with Vm39 (98.3%), and VmTus8
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11 186 with Vm43 (97.3%). The most frequent sequence variant identified in Tuscany was VmTus3
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13 187 (52.9% of the BNP strains), followed by VmTus6 (11.8%), VmTus1 and VmTus2 (8.8% for each),
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15 188 VmTus4 and VmTus5 (5.9% for each), VmTus7 and VmTus8 (2.9% for each) (Table 2). For each
16
17 189 *vmp1* sequence variant, one representative nucleotide sequence was deposited to NCBI GenBank at
18
19 190 Accession Number shown in Table 2 (named from VmTus1 to VmTus8).

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22 191 The 63 *stamp* fragments (StampF1/R1) of the expected size were sequenced (Table 1). Based
23
24 192 on sequence identity, 5 different *stamp* sequence variants (StTus1 to StTus5) were identified (Table
25
26 193 3). Comparison of such sequence variants (from StTus1 to StTus5) with the updated dataset (Table
27
28 194 S2) revealed that they were identical to the previously described sequence variants St5, St9, St10,
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30 195 St11, and St18. The most frequent sequence variant among BNP strains identified in Tuscany was
31
32 196 StTus3 (46.0%), followed by StTus1 (34.9%), StTus5 (12.7%), StTus4 (4.8%), and StTus2 (1.6%)
33
34 197 (Table 3). One representative *stamp* nucleotide sequence variant was deposited to NCBI GenBank
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36 198 at Accession Number shown in Table 3.

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38
39 199 Based on the combination of *vmp1* and *stamp* sequence variants, BNP strains identified in
40
41 200 Tuscany were attributed to 14 *vmp1/stamp* genotypes (Table 1, 4). The most abundant genotype
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43 201 identified in the present study was VmTus3(Vm43)/StTus3(St10), previously reported in the
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45 202 Chianti Classico area (Tuscany, Central Italy) (Pierro *et al.*, 2018).

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49 50 204 **Phylogenetic analysis and selective pressure on BNP strains**

51
52 205 The phylogenetic tree, generated from the alignment of *vmp1* nucleotide sequences of BNP
53
54 206 strains representative of the Vm sequence variants identified in Tuscany (VmTus1 to VmTus8) and
55
56 207 those previously described (Vm1 to Vm87) (Table S1), showed the presence of five main clusters.

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3 208 Sequence variant VmTus2 grouped in the cluster *vmp1*-1, variants VmTus3 and VmTus8 in the
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5 209 cluster *vmp1*-2, VmTus1 and VmTus7 in the cluster *vmp1*-3, VmTus4 and VmTus5 in the cluster
6
7 210 *vmp1*-4, and VmTus6 in the cluster *vmp1*-5 (Figure 1).
8

9 211 The alignment of *stamp* nucleotide sequences of BNP strains representative of the St sequence
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11 212 variants identified in Tuscany (StTus1 to StTus5) and those previously described (St1 to St58)
12
13 213 (Table S2) was used for generating a phylogenetic tree in which four main clusters were observed.
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15 214 '*Ca. P. solani*' strains sharing the *stamp* sequence variant StTus2 grouped in the nettle-related
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17 215 subcluster a2; those with sequence variants StTus4 and StTus5 grouped in the nettle-related
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19 216 subcluster a1; those with sequence variant StTus1 grouped in the bindweed-related cluster b-II;
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21 217 those with sequence variant StTus3 in the bindweed-related cluster b-I (Figure 2).
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24 218 The phylogenetic tree, generated from the alignment of *vmp1/stamp* concatenated nucleotide
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26 219 sequences of BNP strains representative of the *vmp1/stamp* types identified in Tuscany (Table 2, 3,
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28 220 4) and those previously described (Vm10/St2 to Vm76/St1) (Table S3), showed the presence of five
29
30 221 main clusters. Three *vmp1/stamp* types (VmTus2/StTus1, VmTus2/StTus3, and VmTus2/StTus5)
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32 222 grouped in cluster *vmp1/stamp*-1; four *vmp1/stamp* types (VmTus4/StTus1, VmTus4/StTus4,
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34 223 VmTus5/StTus1, and VmTus5/StTus5) grouped in the cluster *vmp1/stamp*-2; three *vmp1/stamp*
35
36 224 types (VmTus3/StTus1, VmTus3/StTus3, and VmTus8/StTus3) grouped in the cluster *vmp1/stamp*-
37
38 225 3; two *vmp1/stamp* types (VmTus1/StTus1 and VmTus7/StTus1) grouped in the cluster
39
40 226 *vmp1/stamp*-4; two *vmp1/stamp* types (VmTus6/StTus1 and VmTus6/StTus3) grouped in the
41
42 227 cluster *vmp1/stamp*-5 (Figure 3, Table 4). Further analyses revealed a difference between intra-
43
44 228 cluster and inter-cluster genetic heterogeneity of BNP strains, calculated on both nucleotide and
45
46 229 amino acid sequence alignments. In detail, BNP strains within each cluster (intra-cluster
47
48 230 heterogeneity) shared a mean nucleotide/amino acid sequence identity of 98.4%/97.1%
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50 231 (approximately 29 SNPs/17 amino acid positions distinguishing one strain to another); on the other
51
52 232 hand, BNP strains of distinct clusters (inter-cluster heterogeneity) shared a mean nucleotide/amino
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54 233 acid sequence identity of 95.1%/90.3% (approximately 90 SNPs/59 amino acid positions
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3 234 distinguishing one strain to another) (Table S4). Chi square test analyses showed statistically
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5 235 significant differences in the distribution of BNP strains, grouped in distinct *vmp1/stamp* clusters, in
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7 236 north-western (Lucca and Massa districts) and central-eastern (Arezzo, Firenze, and Siena districts)
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9 237 Tuscany ($p = 0.006$) (Figure 4).
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11 238

13 239 **DISCUSSION**

15 240 Multi-locus sequence typing (MLST) analysis based on the *stamp* and *vmp1* genes, that are
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17 241 strictly correlated to biological features of '*Ca. Phytoplasma solani*' strains (Langer & Maixner,
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19 242 2004; Murolo & Romanazzi, 2015; Kosovac *et al.*, 2016; Pierro *et al.*, 2018), contributes to the
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21 243 understanding of the ecology of this pathogen.
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24 244 Considering the attribution of '*Ca. P. solani*' strains to different epidemiological systems based
25
26 245 on their grouping in distinct *stamp* phylogenetic clusters (Atanasova *et al.*, 2015), the obtained
27
28 246 results confirmed that BNP ecology in Tuscan vineyards is mainly associated to the bindweed-
29
30 247 related host system (81.0% of BNP strains grouped in clusters b-I and b-II), but revealed a lesser
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32 248 role of the nettle-related host system (19.0% of BNP strains grouped in subclusters a1 and a2).
33
34 249 These results are in accordance with previous studies carried out in central and southern Italy
35
36 250 (Pacifico *et al.*, 2007; Marchi *et al.*, 2015; Murolo & Romanazzi 2015; Pierro *et al.*, 2018).
37

39 251 MLST analyses allowed the identification of 14 BNP *vmp1/stamp* collective genotypes: 11
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41 252 showing *stamp* sequence variants StTus1 (St5) and StTus3 (St10), associated with bindweed-related
42
43 253 host system, and three showing *stamp* sequence variants StTus4 (St11) and StTus5 (St18),
44
45 254 associated with nettle-related host system. Within BNP strains associated with the bindweed-related
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47 255 host system, the results evidenced the prevalence of the genotype Vm43/St10 (35.5% of collective
48
49 256 BNP genotypes described in Tuscany) in Tuscan vineyards (Table 4), extending to Regional level
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51 257 the results previously obtained by Pierro *et al.* (2018) in a case study vineyard in Greve in Chianti,
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53 258 district of Firenze. Interestingly, based on sequence identity and phylogenetic clustering obtained by
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55 259 the analysis of *vmp1*, *stamp* and concatenated *vmp1/stamp* nucleotide sequences, this prevalent BNP
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3 260 genotype was strictly related to '*Ca. P. solani*' strains previously found mainly in Solanaceae hosts
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5 261 and in insect vectors (*H. obsoletus* and *R. quinquecostatus*) in France and central Italy (Cimerman
6
7 262 *et al.*, 2009; Murolo & Romanazzi, 2015; Landi *et al.*, 2015; Chuche *et al.*, 2016), but never in
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9 263 grapevines outside of Tuscany (Pierro *et al.*, 2018). The remaining ten BNp genotypes were
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11 264 sporadically found and represent, when considered all together, 55% of BNp genotypes described in
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13 265 Tuscany (Table 4). Based on sequence identities and grouping in phylogenetic clusters obtained by
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15 266 the analysis of *vmp1*, *stamp* and concatenated *vmp1/stamp* nucleotide sequences, such BNp
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17 267 genotypes were closely related to those largely found in association with plant hosts and insect
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19 268 vectors in Euro-Mediterranean countries (Figure 1, 2, 3). Within BNp strains associated with the
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21 269 nettle-related host system, the results evidenced that the three collective BNp genotypes were
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23 270 sporadically found and represented, when considered all together, 10% of BNp genotypes described
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25 271 in Tuscany (Table 4). Sequence identity and phylogenetic analyses of *vmp1*, *stamp* and
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27 272 concatenated *vmp1/stamp* nucleotide sequences confirmed the strict relationship between these
28
29 273 Tuscan BNp genotypes and those previously found in association with nettle-related host system in
30
31 274 Europe (Figure 1, 2, 3). Based on these evidences, it is reasonable to propose that BN epidemiology
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33 275 in Tuscany involves (i) two BNp strain populations related to bindweed and nettle host systems
34
35 276 playing a pivotal role in BNp diffusion in European vineyards, and (ii) a BNp strain population,
36
37 277 putatively associated with bindweed (*stamp* cluster b-I, but never found directly in *C. arvensis*),
38
39 278 including strains (mostly genotype Vm43/St10) identified in vineyard agro-ecosystems exclusively
40
41 279 in Tuscany. Such BNp strains are similar to '*Ca. Phytoplasma solani*' strains previously detected in
42
43 280 Solanaceae, *H. obsoletus* and *R. quinquecostatus* in Europe. Future studies will focus on
44
45 281 investigating the epidemiological patterns of those BNp strains through the identification of their
46
47 282 putative insect vector(s) and plant host(s) in Tuscan vineyard agro-ecosystems.

52 283 Environmental and ecological features in agro-ecosystems in distinct geographic areas may be
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54 284 significant, directly or indirectly, in determining the strain composition of phytoplasma populations
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56 285 in different regions (Cai *et al.*, 2008; Wu *et al.*, 2012; Quaglino *et al.*, 2017). In the present study,

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3 286 after having described the genetic variability among BNP strain populations in Tuscan vineyards,
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5 287 we investigated whether the composition of BNP strain populations was influenced by the
6
7 288 geographic origin of the samples (north-western Tuscany, including Massa and Lucca districts;
8
9 289 central-eastern Tuscany, including Arezzo, Firenze and Siena districts). The use of the geographic
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11 290 origin as a variable for the statistical analyses revealed a significant difference in the BNP strain
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13 291 distribution based on *vmp1/stamp* clusters between the two areas (Figure 4). This result will need
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15 292 further confirmation based on the analysis of larger datasets considering the high variability of the
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17 293 analyzed genes.

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20 294 The overall findings of the present study reinforced the hypothesis that distinct geographic
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22 295 areas, probably associated with different ecological niches, can play a role, directly (*i.e.* selection of
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24 296 fittest phytoplasma strains) or indirectly (*i.e.* influence on wild plant hosts and insect vectors), in
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26 297 shaping the composition of BNP strain populations. Interestingly, the prevalence in symptomatic
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28 298 grapevines of a '*Ca. P. solani*' genotype (Vm43/St10), which was so far not associated with
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30 299 grapevine but with different hosts in other geographic regions, should be considered to better
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32 300 understand the epidemiology of the disease in the future.

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Supporting Information

Pierro *et al.* [Prevalence of unusual '*Ca. P. solani*' strain in Tuscan grapevines]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. <i>vmp1</i>
Vm1	S13	<i>Vitis vinifera</i>	Italy	HM008616
Vm2	Neuweier57_C	<i>Convolvulus arvensis</i>	Germany	JQ977736
Vm3	C_Boppard_C	<i>Convolvulus arvensis</i>	Germany	JQ977734
Vm4	N10	<i>Urtica dioica</i>	France	JQ977730
Vm5	N9	<i>Urtica dioica</i>	France	JQ977729
Vm6	N2	<i>Urtica dioica</i>	Germany	JQ977722
Vm7	N1	<i>Urtica dioica</i>	Germany	JQ977721
Vm8	CH1	<i>Vitis vinifera</i>	Italy	AM992105
Vm8	T2_56	<i>Solanum lycopersicum</i>	Italy	AM992104
Vm9	P74/11	<i>Vitis vinifera</i>	Italy	KJ145361
Vm10	Vv17	<i>Vitis vinifera</i>	Serbia	KC703032
Vm10	Vv21	<i>Vitis vinifera</i>	Serbia	KC703026
Vm10	Vexp Rpm5	<i>Reptalus panzeri</i>	Serbia	KC703028
Vm10	Vexp Rpg11	<i>Reptalus panzeri</i>	Serbia	KC703027
Vm10	Rpm34	<i>Reptalus panzeri</i>	Serbia	KC703024
Vm10	Rpg39	<i>Reptalus panzeri</i>	Serbia	KC703023
Vm10	Rqg31	<i>Reptalus quinquecostatus</i>	Serbia	KC703031
Vm10	Rqg60	<i>Reptalus quinquecostatus</i>	Serbia	KC703025
Vm10	STOL	<i>Capsicum annuum</i>	Serbia	AM992103
Vm11	LA6_I_C	<i>Convolvulus arvensis</i>	Germany	JQ977735
Vm12	GGY	<i>Vitis vinifera</i>	Germany	AM992102
Vm13	MK29	<i>Vitis vinifera</i>	Macedonia	KF957604
Vm14	Vv12_754	<i>Vitis vinifera</i>	Austria	KJ469734
Vm14	Vv12_751	<i>Vitis vinifera</i>	Austria	KJ469734
Vm14	Vv12_Kn6	<i>Vitis vinifera</i>	Austria	KJ469734
Vm15	60/11	<i>Vitis vinifera</i>	Italy	KJ145346
Vm15	Aa25	<i>Vitis vinifera</i>	Italy	HM008614
Vm15	Mri10	<i>Vitis vinifera</i>	Italy	HM008615
Vm15	HY.86N	<i>Hyalesthes obsoletus</i>	Italy	KM225871
Vm15	HY.80N	<i>Hyalesthes obsoletus</i>	Italy	KM225870
Vm15	Ne.10	<i>Urtica dioica</i>	Italy	KM225869
Vm15	Ho13_1006	<i>Hyalesthes obsoletus</i>	Austria	KJ469727
Vm16	N13	<i>Urtica dioica</i>	Italy	JQ977733
Vm17	N12	<i>Urtica dioica</i>	Italy	JQ977732
Vm18	N11	<i>Urtica dioica</i>	Italy	JQ977731
Vm18	Ho13_838	<i>Hyalesthes obsoletus</i>	Austria	KJ469729
Vm19	N8	<i>Urtica dioica</i>	Italy	JQ977728
Vm20	N7	<i>Urtica dioica</i>	Italy	JQ977727
Vm21	N6	<i>Urtica dioica</i>	Italy	JQ977726
Vm22	N5	<i>Urtica dioica</i>	Italy	JQ977725
Vm23	CrHo13_1183	<i>Hyalesthes obsoletus</i>	Austria	KJ469728
Vm23	N4	<i>Urtica dioica</i>	Slovenia	JQ977724
Vm24	N3	<i>Urtica dioica</i>	Slovenia	JQ977723
Vm25	MK44	<i>Vitis vinifera</i>	Macedonia	KF957605
Vm26	149/11	<i>Vitis vinifera</i>	Italy	KJ145347
Vm27	Aa16	<i>Vitis vinifera</i>	Italy	HM008602
Vm27	Bi.15	<i>Convolvulus arvensis</i>	Italy	KM225875
Vm27	HY.9B	<i>Hyalesthes obsoletus</i>	Italy	KM225874
Vm28	166/11	<i>Vitis vinifera</i>	Italy	KJ145355
Vm28	136/11	<i>Vitis vinifera</i>	Italy	KJ145354
Vm28	P10/11	<i>Vitis vinifera</i>	Italy	KJ145353
Vm28	Aaq1	<i>Vitis vinifera</i>	Italy	HM008601

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443 **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. <i>vmp1</i>
Vm28	Mca21	<i>Vitis vinifera</i>	Italy	HM008599
Vm28	B51	<i>Vitis vinifera</i>	Italy	HM008600
Vm28	Rpg47	<i>Reptalus panzeri</i>	Serbia	KC703034
Vm28	Rqg50	<i>Reptalus quinquecostatus</i>	Serbia	KC703033
Vm28	D_Bacharach_C	<i>Convolvulus arvensis</i>	Germany	JQ977738
Vm28	MK28	<i>Vitis vinifera</i>	Macedonia	KF957603
Vm28	CrAr12_722_2	<i>Anaceratagallia ribauti</i>	Austria	KJ469735
Vm28	Vv12_752	<i>Vitis vinifera</i>	Austria	KJ469735
Vm28	17-11	<i>Vitis vinifera</i>	Bosnia & Herzegovina	KP739858
Vm29	M33_F_C	<i>Convolvulus arvensis</i>	France	JQ977742
Vm30	EisHo1_C	<i>Convolvulus arvensis</i>	Italy	JQ977740
Vm31	Charente-1	<i>Hyalesthes obsoletus</i>	France	AM992098
Vm32	Moliere	<i>Prunus avium</i>	France	AM992096
Vm33	CrHo12_601	<i>Hyalesthes obsoletus</i>	Austria	KJ469730
Vm34	19-25	<i>Vitis vinifera</i>	Germany	AM992101
Vm35	PO	<i>Hyalesthes obsoletus</i>	France	AM992095
Vm36	Rqg42	<i>Reptalus quinquecostatus</i>	Serbia	KC703030
Vm37	SFRT1	<i>Vitis vinifera</i>	Italy	KJ129606
Vm38	LG	<i>Solanum lycopersicum</i>	France	AM992097
Vm39	78/11	<i>Vitis vinifera</i>	Italy	KJ145349
Vm39	B7	<i>Vitis vinifera</i>	Italy	HM008608
Vm39	HY.31B	<i>Hyalesthes obsoletus</i>	Italy	KM225862
Vm39	HY.24B	<i>Hyalesthes obsoletus</i>	Italy	KM225861
Vm39	San31_2016	<i>Vitis vinifera</i>	Italy	MF182859
Vm40	Mp49	<i>Vitis vinifera</i>	Italy	HM008607
Vm41	B2035	<i>Vitis vinifera</i>	Italy	HM008611
Vm41	C1	<i>Vitis vinifera</i>	Italy	HM008610
Vm41	San47_2016	<i>Vitis vinifera</i>	Italy	MF182860
Vm42	Mca28	<i>Vitis vinifera</i>	Italy	HM008609
Vm42	San24_2016	<i>Vitis vinifera</i>	Italy	MF182861
Vm43	ARSIA1	<i>Linaria vulgaris</i>	Italy	KJ129605
Vm43	HY.3B	<i>Hyalesthes obsoletus</i>	Italy	KM225877
Vm43	HY32.B	<i>Hyalesthes obsoletus</i>	Italy	KM225876
Vm43	San21_2015	<i>Vitis vinifera</i>	Italy	MF182856
Vm44	Mvercer2	<i>Vitis vinifera</i>	Italy	HM008612
Vm45	San2_2015	<i>Vitis vinifera</i>	Italy	MF182857
Vm45	315/11	<i>Vitis vinifera</i>	Italy	KJ145360
Vm45	P136/11	<i>Vitis vinifera</i>	Italy	KJ145358
Vm45	P75/11	<i>Vitis vinifera</i>	Italy	KJ145357
Vm45	411/11	<i>Vitis vinifera</i>	Italy	KJ145359
Vm45	Bi.47	<i>Convolvulus arvensis</i>	Italy	KM225881
Vm45	HY.48N	<i>Hyalesthes obsoletus</i>	Italy	KM225880
Vm45	HY.50B	<i>Hyalesthes obsoletus</i>	Italy	KM225879
Vm46	353/11	<i>Vitis vinifera</i>	Italy	KJ145352
Vm46	287/11	<i>Vitis vinifera</i>	Italy	KJ145351
Vm46	115/11	<i>Vitis vinifera</i>	Italy	KJ145350
Vm46	Mp46	<i>Vitis vinifera</i>	Italy	HM008606
Vm46	Ag4a	<i>Vitis vinifera</i>	Italy	HM008605
Vm46	Bi.13	<i>Convolvulus arvensis</i>	Italy	KM225866
Vm46	Vv24	<i>Vitis vinifera</i>	Serbia	KC703036
Vm46	Vv5	<i>Vitis vinifera</i>	Serbia	KC703035
Vm46	Rpm35	<i>Reptalus panzeri</i>	Serbia	KC703029
Vm46	PM1	<i>Solanum tuberosum</i>	Montenegro	KU588192
Vm47	B49	<i>Vitis vinifera</i>	Italy	HM008604
Vm48	C3	<i>Vitis vinifera</i>	Italy	HM008603
Vm49	MK19	<i>Vitis vinifera</i>	Macedonia	KF957602

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446 **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. <i>vmp1</i>
Vm50	HY.14B	<i>Hyalesthes obsoletus</i>	Italy	KM225865
Vm50	HY.5B	<i>Hyalesthes obsoletus</i>	Italy	KM225864
Vm50	HY.12B	<i>Hyalesthes obsoletus</i>	Italy	KM225863
Vm50	Ca13_RF	<i>Convolvulus arvensis</i>	Austria	KJ469732
Vm51	C6	<i>Vitis vinifera</i>	Italy	HM008618
Vm51	B4	<i>Vitis vinifera</i>	Italy	HM008617
Vm51	RA6_I_C	<i>Convolvulus arvensis</i>	Italy	JQ977737
Vm52	I_Norheim_C	<i>Convolvulus arvensis</i>	Germany	JQ977739
Vm52	Charente-2	<i>Hyalesthes obsoletus</i>	France	AM992099
Vm53	P7	<i>Catharanthus roseus</i>	Lebanon	AM992100
Vm53	Tsol89	<i>Vitis vinifera</i>	Georgia	KT184878
Vm53	Kiqu94	<i>Vitis vinifera</i>	Georgia	KT184878
Vm54	P42/11	<i>Vitis vinifera</i>	Italy	KJ145356
Vm55	T2_92	<i>Solanum lycopersicum</i>	Italy	AM992106
Vm56	36861_SLO_C	<i>Convolvulus arvensis</i>	Slovenia	JQ977741
Vm57	CrHo12_721	<i>Hyalesthes obsoletus</i>	Austria	KJ469731
Vm58	Mag1	<i>Vitis vinifera</i>	Italy	HM008613
Vm58	HY.7N	<i>Hyalesthes obsoletus</i>	Italy	KM225868
Vm58	HY.18N	<i>Hyalesthes obsoletus</i>	Italy	KM225867
Vm59	MK94	<i>Vitis vinifera</i>	Macedonia	KF957606
Vm60	CrHo12_650	<i>Hyalesthes obsoletus</i>	Austria	KJ469725
Vm61	Vv12_274	<i>Vitis vinifera</i>	Austria	KJ469726
Vm62	425/11	<i>Vitis vinifera</i>	Italy	KJ145348
Vm63	Vv12_III6	<i>Vitis vinifera</i>	Austria	KJ469733
Vm64	Carv1	<i>Convolvulus arvensis</i>	Georgia	KT184867
Vm65	Carv2	<i>Convolvulus arvensis</i>	Georgia	KT184868
Vm66	Char7	<i>Vitis vinifera</i>	Georgia	KT184869
Vm67	Char8	<i>Vitis vinifera</i>	Georgia	KT184870
Vm68	Sape19	<i>Vitis vinifera</i>	Georgia	KT184871
Vm69	GoMt25	<i>Vitis vinifera</i>	Georgia	KT184872
Vm70	Kisi38	<i>Vitis vinifera</i>	Georgia	KT184873
Vm71	Rkat47	<i>Vitis vinifera</i>	Georgia	KT184874
Vm71	Sape51	<i>Vitis vinifera</i>	Georgia	KT184874
Vm71	Sape62	<i>Vitis vinifera</i>	Georgia	KT184874
Vm72	Khik70	<i>Vitis vinifera</i>	Georgia	KT184875
Vm73	Amla77	<i>Vitis vinifera</i>	Georgia	KT184876
Vm74	Sabu84	<i>Vitis vinifera</i>	Georgia	KT184877
Vm75	LN-b	<i>Salvia miltiorrhiza</i>	China	KU600116
Vm75	LN-a	<i>Salvia miltiorrhiza</i>	China	KU600115
Vm75	LY-6	<i>Salvia miltiorrhiza</i>	China	KU600114
Vm75	LY-5	<i>Salvia miltiorrhiza</i>	China	KU600113
Vm75	LY-4	<i>Salvia miltiorrhiza</i>	China	KU600112
Vm75	SZ-9	<i>Salvia miltiorrhiza</i>	China	KU600111
Vm75	SZ-8	<i>Salvia miltiorrhiza</i>	China	KU600110
Vm75	SZ-7	<i>Salvia miltiorrhiza</i>	China	KU600109
Vm75	LN-3	<i>Salvia miltiorrhiza</i>	China	KU600108
Vm75	LN-2	<i>Salvia miltiorrhiza</i>	China	KU600107
Vm75	LN-1	<i>Salvia miltiorrhiza</i>	China	KU600106
Vm76	G24-13	<i>Vitis vinifera</i>	Bosnia & Herzegovina	KT766176
Vm76	05-09	<i>Vitis vinifera</i>	Bosnia & Herzegovina	KT766163
Vm76	PM2	<i>Solanum tuberosum</i>	Montenegro	KU588193
Vm77	12-11	<i>Vitis vinifera</i>	Bosnia & Herzegovina	KT766169
Vm78	HY.8B	<i>Hyalesthes obsoletus</i>	Italy	KM225878
Vm79	HY.6B	<i>Hyalesthes obsoletus</i>	Italy	KM225873
Vm79	HY.25B	<i>Hyalesthes obsoletus</i>	Italy	KM225872
Vm80	Bi.2	<i>Convolvulus arvensis</i>	Italy	KM225860
Vm80	HY.53B	<i>Hyalesthes obsoletus</i>	Italy	KM225859
Vm81	San24_2015	<i>Vitis vinifera</i>	Italy	MF182858

448 **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. <i>vmp1</i>
Vm82	San49_2016	<i>Vitis vinifera</i>	Italy	MF182862
Vm83	San11_2016	<i>Vitis vinifera</i>	Italy	MF182863
Vm84	San37_2016	<i>Vitis vinifera</i>	Italy	MF182864
Vm85	San5_2016	<i>Vitis vinifera</i>	Italy	MF182865
Vm86	San56_2016	<i>Vitis vinifera</i>	Italy	MF182866
Vm87	San43_2016	<i>Vitis vinifera</i>	Italy	MF182867

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Supporting Information

Pierro *et al.* [Prevalence of unusual 'Ca. P. solani' strain in Tuscan grapevines]Table S2. Sequence variants of the gene *stamp* among 'Ca. P. solani' strains available in GenBank (part I)

Sequence variant	Strain	Host	Location	Acc. N. <i>stamp</i>
St1	Rqg50	<i>Reptalus quinquecostatus</i>	Serbia	KC703019
St1	11-11	<i>Vitis vinifera</i>	Bosnia & Herzegovina	KP739852
St1	115/11	<i>Vitis vinifera</i>	Italy	KJ145337
St1	17-11	<i>Vitis vinifera</i>	Bosnia & Herzegovina	KP739854
St1	20MN	<i>Vitis vinifera</i>	Montenegro	KJ926068
St1	353/11	<i>Vitis vinifera</i>	Italy	KJ145338
St1	45MN	<i>Convolvulus arvensis</i>	Montenegro	KJ926069
St1	72MN	<i>Vitex agnus-castus</i>	Montenegro	KJ926070
St1	Ag4a	<i>Vitis vinifera</i>	Italy	KJ145377
St1	B1	<i>Vitis vinifera</i>	Italy	KJ145378
St1	C45	<i>Convolvulus arvensis</i>	Macedonia	KP337319
St1	CrAr12_722_2	<i>Anaceratagallia ribauti</i>	Austria	KJ469722
St1	CrHo12_721	<i>Hyalesthes obsoletus</i>	Austria	KJ469722
St1	G21-13	<i>Vitis vinifera</i>	Bosnia & Herzegovina	KP739856
St1	G22-13	<i>Vitis vinifera</i>	Bosnia & Herzegovina	KP739849
St1	G23-13	<i>Vitis vinifera</i>	Bosnia & Herzegovina	KP739846
St1	G24-13	<i>Vitis vinifera</i>	Bosnia & Herzegovina	KP739847
St1	G4-13	<i>Vitis vinifera</i>	Bosnia & Herzegovina	KP739853
St1	G6-13	<i>Vitis vinifera</i>	Bosnia & Herzegovina	KP739848
St1	Gb1	<i>Phaseolus vulgaris</i>	Serbia	KM977907
St1	Ho375	<i>Hyalesthes obsoletus</i>	Montenegro	KJ926071
St1	Ho66-2	<i>Hyalesthes obsoletus</i>	Montenegro	KJ926072
St1	HoC202	<i>Hyalesthes obsoletus</i>	Macedonia	KP337320
St1	Mp46	<i>Vitis vinifera</i>	Italy	KJ145379
St1	P25/11	<i>Vitis vinifera</i>	Italy	KJ145339
St1	PM1	<i>Solanum tuberosum</i>	Montenegro	KU588188
St1	PM2	<i>Solanum tuberosum</i>	Montenegro	KU588189
St1	PS8	<i>Solanum tuberosum</i>	Serbia	KP877599
St1	PS8Ho	<i>Hyalesthes obsoletus</i>	Serbia	KP877600
St1	PS8Rp	<i>Reptalus panzeri</i>	Serbia	KP877601
St1	PS9	<i>Solanum tuberosum</i>	Serbia	KP877602
St1	Rpg47	<i>Reptalus panzeri</i>	Serbia	KC703020
St1	Vv12_III6	<i>Vitis vinifera</i>	Austria	KJ469722
St1	Vv5	<i>Vitis vinifera</i>	Serbia	KC703021
St2	Rqg31	<i>Reptalus quinquecostatus</i>	Serbia	KC703017
St2	Br8	<i>Convolvulus arvensis</i>	Croatia	KJ573597
St2	C2_Rgg50	<i>Apium graveolens</i>	Bosnia & Herzegovina	KU295506
St2	Ho41-2	<i>Hyalesthes obsoletus</i>	Montenegro	KJ926065
St2	P10	<i>Capsicum annuum</i>	Bosnia & Herzegovina	KU295504
St2	P6	<i>Capsicum annuum</i>	Bosnia & Herzegovina	KU295502
St2	PS4	<i>Solanum tuberosum</i>	Serbia	KP877588
St2	PS4Ho	<i>Hyalesthes obsoletus</i>	Serbia	KP877589
St2	PS5	<i>Solanum tuberosum</i>	Serbia	KP877590
St2	PS5Ho	<i>Hyalesthes obsoletus</i>	Serbia	KP877591
St2	PS5Rp	<i>Reptalus panzeri</i>	Serbia	KP877592
St2	PS6	<i>Solanum tuberosum</i>	Serbia	KP877593
St2	PS6Ho	<i>Hyalesthes obsoletus</i>	Serbia	KP877594
St2	PS6Rq	<i>Reptalus quinquecostatus</i>	Serbia	KP877595
St2	Vv12_Kn6	<i>Vitis vinifera</i>	Austria	KJ469724
St2	Vv17	<i>Vitis vinifera</i>	Serbia	KC703018
St3	16MN	<i>Vitis vinifera</i>	Montenegro	KJ926073
St3	30-09	<i>Vitis vinifera</i>	Bosnia & Herzegovina	KP739851
St3	43MN	<i>Convolvulus arvensis</i>	Montenegro	KJ926074

474 **Table S2.** Sequence variants of the gene *stamp* among '*Ca. P. solani*' strains available in GenBank (part II)

Sequence variant	Strain	Host	Location	Acc. N. <i>stamp</i>
St3	79MN	<i>Vitex agnus-castus</i>	Montenegro	KJ926075
St3	Ho389	<i>Hyalesthes obsoletus</i>	Montenegro	KJ926076
St3	MK66	<i>Vitis vinifera</i>	Macedonia	KF957608
St3	P5	<i>Capsicum annuum</i>	Bosnia & Herzegovina	KU295501
St3	P7	<i>Catharanthus roseus</i>	Lebanon	FN813258
St3	PS7	<i>Solanum tuberosum</i>	Serbia	KP877596
St3	PS7Ho	<i>Hyalesthes obsoletus</i>	Serbia	KP877597
St3	PS7Rp	<i>Reptalus panzeri</i>	Serbia	KP877598
St3	Rpm35	<i>Reptalus panzeri</i>	Serbia	KC703015
St3	Vv12_751	<i>Vitis vinifera</i>	Austria	KJ469723
St4	G2	<i>Vitis vinifera</i>	Macedonia	KP337318
St4	GR328	<i>Capsicum annuum</i>	Greece	FN813253
St4	Ho10-2	<i>Hyalesthes obsoletus</i>	Montenegro	KJ926067
St4	MB11	<i>Zea mays</i>	Bosnia & Herzegovina	KU295509
St4	MB4	<i>Zea mays</i>	Bosnia & Herzegovina	KU295507
St4	MB6	<i>Zea mays</i>	Bosnia & Herzegovina	KU295508
St4	PS1	<i>Solanum tuberosum</i>	Serbia	KP877583
St4	PS1Rp	<i>Reptalus panzeri</i>	Serbia	KP877584
St4	PS1Rq	<i>Reptalus quinquecostatus</i>	Serbia	KP877585
St4	Rpg39	<i>Reptalus panzeri</i>	Serbia	KC703009
St4	Rpm34	<i>Reptalus panzeri</i>	Serbia	KC703010
St4	Rqg60	<i>Reptalus quinquecostatus</i>	Serbia	KC703011
St4	STOL	<i>Capsicum annuum</i>	Serbia	FN813261
St4	Vexp Rpg11	<i>Reptalus panzeri</i>	Serbia	KC703013
St4	Vexp Rpm5	<i>Reptalus panzeri</i>	Serbia	KC703014
St4	Vv21	<i>Vitis vinifera</i>	Serbia	KC703012
St5	215/11	<i>Vitis vinifera</i>	Italy	KJ145329
St5	287/11	<i>Vitis vinifera</i>	Italy	KJ145332
St5	315/11	<i>Vitis vinifera</i>	Italy	KJ145330
St5	425/11	<i>Vitis vinifera</i>	Italy	KJ145335
St5	78/11	<i>Vitis vinifera</i>	Italy	KJ145334
St5	Ca13_RF	<i>Convolvulus arvensis</i>	Austria	KJ469721
St5	CrHo12_601	<i>Hyalesthes obsoletus</i>	Austria	KJ469721
St5	GGY	<i>Vitis vinifera</i>	Germany	FN813256
St5	HoC205	<i>Hyalesthes obsoletus</i>	Macedonia	KP337315
St5	LA6_I_C	<i>Convolvulus arvensis</i>	Germany	JQ977720
St5	NGA9	<i>Hyalesthes obsoletus</i>	Slovenia	FN813262
St5	P136/11	<i>Vitis vinifera</i>	Italy	KJ145336
St5	P51/11	<i>Vitis vinifera</i>	Italy	KJ145331
St5	P75/11	<i>Vitis vinifera</i>	Italy	KJ145333
St5	Vv12_752	<i>Vitis vinifera</i>	Austria	KJ469721
St5	Vv12_754	<i>Vitis vinifera</i>	Austria	KJ469721
St5	San23_2015	<i>Vitis vinifera</i>	Italy	MF182869
St6	MK44	<i>Vitis vinifera</i>	Macedonia	KF957607
St6	S7	<i>Urtica dioica</i>	Slovenia	JQ977719
St7	S6	<i>Urtica dioica</i>	Italy	JQ977718
St8	49MN	<i>Urtica dioica</i>	Montenegro	KJ926078
St8	4MN	<i>Vitis vinifera</i>	Montenegro	KJ926077
St8	BN-Yan1	<i>Vitis vinifera</i>	Italy	KX151182
St8	Ho13_838	<i>Hyalesthes obsoletus</i>	Austria	KJ469720
St8	Ho13-8	<i>Hyalesthes obsoletus</i>	Montenegro	KJ926079
St8	HoU190	<i>Hyalesthes obsoletus</i>	Macedonia	KP337321
St8	S5	<i>Urtica dioica</i>	Italy	JQ977717
St8	SB5	<i>Vitis vinifera</i>	Croatia	FN813266
St9	60/11	<i>Vitis vinifera</i>	Italy	KJ145345
St9	07-11	<i>Vitis vinifera</i>	Croatia	KP274915
St9	Aa25	<i>Vitis vinifera</i>	Italy	KJ145387
St9	Aaq29	<i>Vitis vinifera</i>	Italy	KJ145388

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476 **Table S2.** Sequence variants of the gene *stamp* among '*Ca. P. solani*' strains available in GenBank (part III)

Sequence variant	Strain	Host	Location	Acc. N. <i>stamp</i>
St9	Ho13_1006	<i>Hyalesthes obsoletus</i>	Austria	KJ469718
St9	Mcil	<i>Vitis vinifera</i>	Italy	KJ145385
St9	S2	<i>Urtica dioica</i>	Slovenia	JQ977714
St10	LG	<i>Solanum lycopersicum</i>	France	FN813259
St10	PO	<i>Hyalesthes obsoletus</i>	France	FN813270
St10	San21_2015	<i>Vitis vinifera</i>	Italy	MF182868
St11	19-25	<i>Vitis vinifera</i>	Germany	FN813267
St11	33MN	<i>Vitis vinifera</i>	Montenegro	KJ926080
St11	67MN	<i>Urtica dioica</i>	Montenegro	KJ926081
St11	CrHo12_650	<i>Hyalesthes obsoletus</i>	Austria	KJ469716
St11	E	<i>Hyalesthes obsoletus</i>	Germany	FN813263
St11	G1	<i>Vitis vinifera</i>	Macedonia	KP337322
St11	GBr2	<i>Vitis vinifera</i>	Croatia	KJ573590
St11	GBr4	<i>Vitis vinifera</i>	Croatia	KJ573591
St11	GVu1	<i>Vitis vinifera</i>	Croatia	KJ573592
St11	GVu2	<i>Vitis vinifera</i>	Croatia	KJ573593
St11	H17	<i>Hyalesthes obsoletus</i>	Croatia	KJ573594
St11	H18	<i>Hyalesthes obsoletus</i>	Croatia	KJ573595
St11	H21	<i>Hyalesthes obsoletus</i>	Croatia	KJ573596
St11	Ho36-8	<i>Hyalesthes obsoletus</i>	Montenegro	KJ926082
St11	HoU17	<i>Hyalesthes obsoletus</i>	Macedonia	KP337323
St11	MK94	<i>Vitis vinifera</i>	Macedonia	KF957609
St12	L646	<i>Lavandula angustifolia</i>	France	FN813265
St13	GR13	<i>Vitis vinifera</i>	Greece	FN813264
St14	C	<i>Solanum lycopersicum</i>	France	FN813260
St15	P7	<i>Capsicum annum</i>	Bosnia & Herzegovina	KU295503
St15	Tsol89	<i>Vitis vinifera</i>	Georgia	KT184885
St15	Kiqu84	<i>Vitis vinifera</i>	Georgia	KT184885
St16	H299	<i>Hyalesthes obsoletus</i>	France	FN813254
St16	L973	<i>Lavandula angustifolia</i>	France	FN813255
St17	Ate17	<i>Vitis vinifera</i>	Italy	KJ145386
St18	266/11	<i>Vitis vinifera</i>	Italy	KJ145344
St18	Aaq1	<i>Vitis vinifera</i>	Italy	KJ145383
St18	Mdxsain	<i>Vitis vinifera</i>	Italy	KJ145384
St18	San2_2015	<i>Vitis vinifera</i>	Italy	MF182870
St19	CrHo13_1183	<i>Hyalesthes obsoletus</i>	Austria	KJ469719
St19	S3	<i>Urtica dioica</i>	Slovenia	JQ977715
St20	136/11	<i>Vitis vinifera</i>	Italy	KJ145340
St20	166/11	<i>Vitis vinifera</i>	Italy	KJ145343
St20	Ate7	<i>Vitis vinifera</i>	Italy	KJ145381
St20	Mca21	<i>Vitis vinifera</i>	Italy	KJ145382
St20	P10/11	<i>Vitis vinifera</i>	Italy	KJ145342
St20	P42/11	<i>Vitis vinifera</i>	Italy	KJ145341
St21	Aa16	<i>Vitis vinifera</i>	Italy	KJ145380
St22	Mvercer2	<i>Vitis vinifera</i>	Italy	KJ145375
St22	San24_2015	<i>Vitis vinifera</i>	Italy	MF182871
St23	Lot et Garonne	<i>Solanum lycopersicum</i>	France	FN813257
St24	HoU93	<i>Hyalesthes obsoletus</i>	Macedonia	KP337314
St24	U79	<i>Hyalesthes obsoletus</i>	Macedonia	KP337313
St25	HoU80	<i>Hyalesthes obsoletus</i>	Macedonia	KP337309
St26	G5	<i>Hyalesthes obsoletus</i>	Macedonia	KP337310
St26	HoU85	<i>Hyalesthes obsoletus</i>	Macedonia	KP337311
St27	U70	<i>Urtica dioica</i>	Macedonia	KP337312
St28	HoC68	<i>Hyalesthes obsoletus</i>	Macedonia	KP337316
St28	PS3	<i>Solanum tuberosum</i>	Serbia	KP877587
St29	Vv12_274	<i>Vitis vinifera</i>	Austria	KJ469717
St30	10MN	<i>Vitis vinifera</i>	Montenegro	KJ926066
St30	04-09	<i>Vitis vinifera</i>	Croatia	KP274914

478 **Table S2.** Sequence variants of the gene *stamp* among '*Ca. P. solani*' strains available in GenBank (part IV)

Sequence variant	Strain	Host	Location	Acc. N. <i>stamp</i>
St30	G25	<i>Vitis vinifera</i>	Macedonia	KP337317
St30	PS10Ho	<i>Hyalesthes obsoletus</i>	Serbia	KP877603
St30	PS10Rq	<i>Reptalus quinquecostatus</i>	Serbia	KP877604
St30	Vv24	<i>Vitis vinifera</i>	Serbia	KC703022
St31	BG4560	<i>Vitis vinifera</i>	Bulgaria	FN813252
St31	PS2	<i>Solanum tuberosum</i>	Serbia	KP877586
St31	Rqg42	<i>Reptalus quinquecostatus</i>	Serbia	KC703016
St32	Mp49	<i>Vitis vinifera</i>	Italy	KJ145376
St33	OSESLO2	<i>Hyalesthes obsoletus</i>	Slovenia	FN813269
St33	Rome15	<i>Hyalesthes obsoletus</i>	Italy	FN813268
St33	S4	<i>Urtica dioica</i>	Italy	JQ977716
St34	S1	<i>Urtica dioica</i>	Germany	JQ977713
St35	Carv1	<i>Convolvulus arvensis</i>	Georgia	KT184879
St36	Carv2	<i>Convolvulus arvensis</i>	Georgia	KT184880
St37	Char7	<i>Convolvulus arvensis</i>	Georgia	KT184881
St37	Kisi38	<i>Vitis vinifera</i>	Georgia	KT184881
St37	Rkat47	<i>Vitis vinifera</i>	Georgia	KT184881
St37	Sape51	<i>Vitis vinifera</i>	Georgia	KT184881
St37	Sape62	<i>Vitis vinifera</i>	Georgia	KT184881
St38	Char8	<i>Convolvulus arvensis</i>	Georgia	KT184882
St38	Sape19	<i>Vitis vinifera</i>	Georgia	KT184882
St38	GoMt25	<i>Vitis vinifera</i>	Georgia	KT184882
St39	Amla77	<i>Vitis vinifera</i>	Georgia	KT184883
St40	Sabu84	<i>Vitis vinifera</i>	Georgia	KT184884
St41	20-set	<i>Vitis vinifera</i>	Bosnia & Herzegovina	KT766177
St42	154-10	<i>Vitis vinifera</i>	Bosnia & Herzegovina	KP739855
St43	03-nov	<i>Vitis vinifera</i>	Bosnia & Herzegovina	KP739850
St44	C1_Rgg35/Rqg31	<i>Apium graveolens</i>	Bosnia & Herzegovina	KU295505
St45	Ho1152	<i>Hyalesthes obsoletus</i>	Montenegro	KM977906
St46	RQ161	<i>Reptalus quinquecostatus</i>	France	LN823951
St47	San3_2015	<i>Vitis vinifera</i>	Italy	MF182872
St48	San4_2015	<i>Vitis vinifera</i>	Italy	MF182873
St49	San6_2015	<i>Vitis vinifera</i>	Italy	MF182874
St50	San10_2015	<i>Vitis vinifera</i>	Italy	MF182875
St51	San22_2015	<i>Vitis vinifera</i>	Italy	MF182876
St52	San6_2016	<i>Vitis vinifera</i>	Italy	MF182877
St53	San8_2016	<i>Vitis vinifera</i>	Italy	MF182878
St54	San16_2016	<i>Vitis vinifera</i>	Italy	MF182879
St55	San17_2016	<i>Vitis vinifera</i>	Italy	MF182880
St56	San28_2016	<i>Vitis vinifera</i>	Italy	MF182881
St57	San29_2016	<i>Vitis vinifera</i>	Italy	MF182882
St58	San45_2016	<i>Vitis vinifera</i>	Italy	MF182883

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Supporting Information

Pierro *et al.* [Prevalence of unusual '*Ca. P. solani*' strain in Tuscan grapevines]Table S3. *vmp1/stamp* types of '*Ca. P. solani*' strains available in GenBank (part I)

<i>vmp1</i>	<i>stamp</i>	Strain	Host	Country
Vm10	St2	Rqg31	<i>Reptalus quinquecostatus</i>	Serbia
Vm10	St2	Vv17	<i>Vitis vinifera</i>	Serbia
Vm10	St4	Rpg39	<i>Reptalus panzeri</i>	Serbia
Vm10	St4	Rpm34	<i>Reptalus panzeri</i>	Serbia
Vm10	St4	Rqg60	<i>Reptalus quinquecostatus</i>	Serbia
Vm10	St4	STOL	<i>Capsicum annum</i>	Serbia
Vm10	St4	Vexp Rpg11	<i>Reptalus panzeri</i>	Serbia
Vm10	St4	Vexp Rpm5	<i>Reptalus panzeri</i>	Serbia
Vm10	St4	Vv21	<i>Vitis vinifera</i>	Serbia
Vm11	St5	LA6_I_C	<i>Convolvulus arvensis</i>	Germany
Vm12	St5	GGY	<i>Vitis vinifera</i>	Germany
Vm14	St2	Vv12_Kn6	<i>Vitis vinifera</i>	Austria
Vm14	St3	Vv12_751	<i>Vitis vinifera</i>	Austria
Vm14	St5	Vv12_754	<i>Vitis vinifera</i>	Austria
Vm15	St9	60/11	<i>Vitis vinifera</i>	Italy
Vm15	St9	Aa25	<i>Vitis vinifera</i>	Italy
Vm15	St9	Ho13_1006	<i>Hyalesthes obsoletus</i>	Austria
Vm18	St8	Ho13_838	<i>Hyalesthes obsoletus</i>	Austria
Vm23	St19	CrHo13_1183	<i>Hyalesthes obsoletus</i>	Austria
Vm25	St6	MK44	<i>Vitis vinifera</i>	Macedonia
Vm27	St21	Aa16	<i>Vitis vinifera</i>	Italy
Vm28	St1	Rqg50	<i>Reptalus quinquecostatus</i>	Serbia
Vm28	St1	17-nov	<i>Vitis vinifera</i>	Bosnia & Herzegovina
Vm28	St1	CrAr12_722_2	<i>Anaceratagallia ribauti</i>	Austria
Vm28	St1	Rpg47	<i>Reptalus panzeri</i>	Serbia
Vm28	St5	Vv12_752	<i>Vitis vinifera</i>	Austria
Vm28	St18	Aaq1	<i>Vitis vinifera</i>	Italy
Vm28	St20	136/11	<i>Vitis vinifera</i>	Italy
Vm28	St20	166/11	<i>Vitis vinifera</i>	Italy
Vm28	St20	Mca21	<i>Vitis vinifera</i>	Italy
Vm28	St20	P10/11	<i>Vitis vinifera</i>	Italy
Vm33	St5	CrHo12_601	<i>Hyalesthes obsoletus</i>	Austria
Vm34	St11	19-25	<i>Vitis vinifera</i>	Germany
Vm35	St10	PO	<i>Hyalesthes obsoletus</i>	France
Vm36	St31	Rqg42	<i>Reptalus quinquecostatus</i>	Serbia
Vm38	St10	LG	<i>Solanum lycopersicum</i>	France
Vm39	St5	78/11	<i>Vitis vinifera</i>	Italy
Vm39	St5	San31_2016	<i>Vitis vinifera</i>	Italy
Vm40	St32	Mp49	<i>Vitis vinifera</i>	Italy
Vm41	St5	San47_2016	<i>Vitis vinifera</i>	Italy
Vm42	St5	San23_2015	<i>Vitis vinifera</i>	Italy
Vm42	St22	San24_2016	<i>Vitis vinifera</i>	Italy
Vm43	St10	San21_2015	<i>Vitis vinifera</i>	Italy
Vm43	St54	San16_2016	<i>Vitis vinifera</i>	Italy
Vm43	St55	San17_2016	<i>Vitis vinifera</i>	Italy
Vm43	St56	San28_2016	<i>Vitis vinifera</i>	Italy
Vm44	St22	Mvercer2	<i>Vitis vinifera</i>	Italy
Vm45	St5	315/11	<i>Vitis vinifera</i>	Italy
Vm45	St5	P136/11	<i>Vitis vinifera</i>	Italy
Vm45	St5	P75/11	<i>Vitis vinifera</i>	Italy
Vm45	St18	San2_2015	<i>Vitis vinifera</i>	Italy
Vm46	St1	115/11	<i>Vitis vinifera</i>	Italy
Vm46	St1	353/11	<i>Vitis vinifera</i>	Italy
Vm46	St1	Ag4a	<i>Vitis vinifera</i>	Italy

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Table S3. *vmp1*/*stamp* types of '*Ca. P. solani*' strains available in GenBank (part II)

<i>vmp1</i>	<i>stamp</i>	Strain	Host	Country
Vm46	St1	Mp46	<i>Vitis vinifera</i>	Italy
Vm46	St1	PM1	<i>Solanum tuberosum</i>	Montenegro
Vm46	St1	Vv5	<i>Vitis vinifera</i>	Serbia
Vm46	St3	Rpm35	<i>Reptalus panzeri</i>	Serbia
Vm46	St5	287/11	<i>Vitis vinifera</i>	Italy
Vm46	St30	Vv24	<i>Vitis vinifera</i>	Serbia
Vm50	St5	Ca13_RF	<i>Convolvulus arvensis</i>	Austria
Vm53	St15	P7	<i>Catharanthus roseus</i>	Lebanon
Vm53	St15	Tsol89	<i>Vitis vinifera</i>	Georgia
Vm53	St15	Kiqu84	<i>Vitis vinifera</i>	Georgia
Vm54	St20	P42/11	<i>Vitis vinifera</i>	Italy
Vm57	St1	CrHo12_721	<i>Hyalesthes obsoletus</i>	Austria
Vm59	St11	MK94	<i>Vitis vinifera</i>	Macedonia
Vm60	St11	CrHo12_650	<i>Hyalesthes obsoletus</i>	Austria
Vm61	St29	Vv12_274	<i>Vitis vinifera</i>	Austria
Vm62	St5	425/11	<i>Vitis vinifera</i>	Italy
Vm63	St1	Vv12_III6	<i>Vitis vinifera</i>	Austria
Vm64	St35	Carv1	<i>Convolvulus arvensis</i>	Georgia
Vm65	St36	Carv2	<i>Convolvulus arvensis</i>	Georgia
Vm66	St37	Char7	<i>Convolvulus arvensis</i>	Georgia
Vm67	St38	Char8	<i>Convolvulus arvensis</i>	Georgia
Vm68	St38	Sape19	<i>Vitis vinifera</i>	Georgia
Vm69	St38	GoMt25	<i>Vitis vinifera</i>	Georgia
Vm70	St37	Kisi38	<i>Vitis vinifera</i>	Georgia
Vm71	St37	Rkat47	<i>Vitis vinifera</i>	Georgia
Vm71	St37	Sape51	<i>Vitis vinifera</i>	Georgia
Vm71	St37	Sape62	<i>Vitis vinifera</i>	Georgia
Vm73	St39	Amla77	<i>Vitis vinifera</i>	Georgia
Vm74	St40	Sabu84	<i>Vitis vinifera</i>	Georgia
Vm76	St1	G24-13	<i>Vitis vinifera</i>	Bosnia & Herzegovina
Vm76	St1	PM2	<i>Solanum tuberosum</i>	Montenegro
Vm81	St22	San24_2015	<i>Vitis vinifera</i>	Italy
Vm82	St18	San49_2016	<i>Vitis vinifera</i>	Italy
Vm83	St18	San11_2016	<i>Vitis vinifera</i>	Italy
Vm84	St5	San37_2016	<i>Vitis vinifera</i>	Italy
Vm85	St10	San5_2016	<i>Vitis vinifera</i>	Italy
Vm86	St10	San56_2016	<i>Vitis vinifera</i>	Italy
Vm87	St5	San35_2016	<i>Vitis vinifera</i>	Italy
Vm87	St18	San43_2016	<i>Vitis vinifera</i>	Italy

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Supporting Information

Pierro *et al.* [Prevalence of unusual '*Ca. P. solani*' strain in Tuscan grapevines]

Table S4. Genetic diversity based on nucleotide (in white) and amino acid (in grey) sequences among *vmp1/stamp* types, identified in the five Tuscan districts, grouped in the same and in distinct phylogenetic clusters

<i>vmp1/stamp</i>		#														
cluster	type		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	VmTus2/StTus1	1	ID	0,983	0,979	0,905	0,882	0,945	0,929	0,902	0,886	0,905	0,895	0,91	0,908	0,893
	VmTus2/StTus3	2	0,992	ID	0,981	0,89	0,884	0,93	0,93	0,886	0,902	0,92	0,88	0,895	0,893	0,908
	VmTus2/StTus5	3	0,99	0,992	ID	0,888	0,892	0,929	0,949	0,885	0,886	0,905	0,878	0,893	0,891	0,893
2	VmTus4/StTus1	4	0,943	0,936	0,933	ID	0,972	0,954	0,934	0,893	0,876	0,885	0,915	0,937	0,947	0,93
	VmTus4/StTus4	5	0,928	0,929	0,933	0,984	ID	0,928	0,938	0,867	0,868	0,877	0,889	0,911	0,921	0,922
	VmTus5/StTus1	6	0,975	0,967	0,965	0,965	0,951	ID	0,979	0,9	0,883	0,922	0,932	0,954	0,944	0,927
3	VmTus5/StTus5	7	0,965	0,967	0,975	0,956	0,956	0,99	ID	0,88	0,881	0,92	0,912	0,934	0,923	0,925
	VmTus3/StTus1	8	0,956	0,949	0,947	0,938	0,923	0,951	0,941	ID	0,982	0,944	0,867	0,889	0,901	0,884
	VmTus3/StTus3	9	0,949	0,956	0,948	0,931	0,925	0,943	0,943	0,992	ID	0,961	0,85	0,872	0,884	0,901
4	VmTus8/StTus3	10	0,96	0,967	0,959	0,932	0,925	0,963	0,962	0,972	0,98	ID	0,883	0,905	0,886	0,903
	VmTus1/StTus1	11	0,95	0,943	0,94	0,947	0,932	0,967	0,957	0,936	0,929	0,942	ID	0,977	0,918	0,901
5	VmTus7/StTus1	12	0,958	0,951	0,948	0,958	0,944	0,978	0,969	0,948	0,94	0,953	0,987	ID	0,93	0,913
	VmTus6/StTus1	13	0,955	0,948	0,946	0,962	0,947	0,971	0,961	0,952	0,945	0,942	0,958	0,965	ID	0,982
	VmTus6/StTus3	14	0,948	0,955	0,947	0,954	0,948	0,963	0,963	0,945	0,952	0,949	0,951	0,958	0,992	ID

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501 Mean % nucleotide sequence identity within clusters: *vmp1/stamp*-1: 99.13; *vmp1/stamp*-2: 96.7; *vmp1/stamp*-3: 98.13; *vmp1/stamp*-4: 98.7; *vmp1/stamp*-5: 99.2.

502 Mean % nucleotide sequence identity of the cluster *vmp1/stamp*-1 vs: *vmp1/stamp*-2: 95.13; *vmp1/stamp*-3: 95.45; *vmp1/stamp*-4: 94.83; *vmp1/stamp*-5: 94.98.

503 Mean % nucleotide sequence identity of the cluster *vmp1/stamp*-2 vs: *vmp1/stamp*-3: 93.97; *vmp1/stamp*-4: 95.65; *vmp1/stamp*-5: 95.86.

504 Mean % nucleotide sequence identity of the cluster *vmp1/stamp*-3 vs: *vmp1/stamp*-4: 94.13; *vmp1/stamp*-5: 94.75.

505 Mean % nucleotide sequence identity of the cluster *vmp1/stamp*-4 vs *vmp1/stamp*-5: 95.80.

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507 Mean % amino acid sequence identity of the cluster *vmp1/stamp*-1: 98.10; *vmp1/stamp*-2: 95.08; *vmp1/stamp*-3: 96.23; *vmp1/stamp*-4: 97.70; *vmp1/stamp*-5: 98.20.

508 Mean % amino acid sequence identity of the cluster *vmp1/stamp*-1 vs: *vmp1/stamp*-2: 91.27; *vmp1/stamp*-3: 89.74; *vmp1/stamp*-4: 89.18; *vmp1/stamp*-5: 89.76.

509 Mean % amino acid sequence identity of the cluster *vmp1/stamp*-2 vs: *vmp1/stamp*-3: 88.76; *vmp1/stamp*-4: 92.30; *vmp1/stamp*-5: 92.98.

510 Mean % amino acid sequence identity of the cluster *vmp1/stamp*-3 vs: *vmp1/stamp*-4: 87.76; *vmp1/stamp*-5: 89.31.

511 Mean % amino acid sequence identity of the cluster *vmp1/stamp*-4 vs *vmp1/stamp*-5: 91.55.

TABLES

Pierro *et al.* [Prevalence of unusual '*Ca. P. solani*' strain in Tuscan grapevines]

Table 1. BNP genotypes, based on *vmp1* and *stamp* genes, identified in Tuscany on *Vitis vinifera* L. cv. Chardonnay and Sangiovese (part I)

BNp strain	Vineyard	Management	District	Cultivar	seq. var.	
					<i>vmp1</i>	<i>stamp</i>
144	Marciano della Chiana	conventional	AR	Chardonnay		
146	Marciano della Chiana	conventional	AR	Chardonnay	VmTus1	StTus1
147	Marciano della Chiana	conventional	AR	Chardonnay		StTus5
151	Marciano della Chiana	conventional	AR	Chardonnay		StTus3
152	Marciano della Chiana	conventional	AR	Chardonnay		
149	Marciano della Chiana	conventional	AR	Sangiovese		StTus3
154	Marciano della Chiana	conventional	AR	Sangiovese		StTus3
155	Marciano della Chiana	conventional	AR	Sangiovese	VmTus2	StTus5
189	Barberino V. Elsa	conventional	FI	Sangiovese		StTus1
190	Barberino V. Elsa	conventional	FI	Chardonnay	VmTus2	StTus1
192	Barberino V. Elsa	conventional	FI	Chardonnay	VmTus7	StTus1
193	Barberino V. Elsa	conventional	FI	Chardonnay	VmTus3	StTus3
194	Barberino V. Elsa	conventional	FI	Chardonnay		StTus3
PI76	Tavernelle Val di Pesa	organic	FI	Chardonnay		
PI77	Tavernelle Val di Pesa	organic	FI	Chardonnay	VmTus3	StTus3
PI78	Tavernelle Val di Pesa	organic	FI	Chardonnay		StTus5
PI79	Tavernelle Val di Pesa	organic	FI	Chardonnay		
184	Lastra a Signa	conventional	FI	Sangiovese	VmTus3	StTus3
185	Lastra a Signa	conventional	FI	Sangiovese	VmTus3	StTus3
186	Lastra a Signa	conventional	FI	Sangiovese	VmTus8	StTus3
187	Lastra a Signa	conventional	FI	Sangiovese	VmTus3	StTus3
188	Lastra a Signa	conventional	FI	Sangiovese	VmTus3	StTus3
180	Lastra a Signa	conventional	FI	Sangiovese		StTus1
115	Lastra a Signa	conventional	FI	Sangiovese	VmTus3	
PI41	Greve in Chianti (I)	conventional	FI	Sangiovese	VmTus1	StTus1
PI42	Greve in Chianti (I)	conventional	FI	Sangiovese	VmTus1	StTus1
PI43	Greve in Chianti (I)	conventional	FI	Sangiovese	VmTus6	StTus1
PI45	Greve in Chianti (I)	conventional	FI	Sangiovese		StTus1
PI47	Greve in Chianti (I)	conventional	FI	Sangiovese	VmTus2	StTus3
PI48	Greve in Chianti (I)	conventional	FI	Sangiovese	VmTus3	StTus1
PI49	Greve in Chianti (I)	conventional	FI	Sangiovese	VmTus3	
PI50	Greve in Chianti (I)	conventional	FI	Sangiovese	VmTus3	
PI56	Greve in Chianti (I)	conventional	FI	Sangiovese	VmTus3	StTus1
PI57	Greve in Chianti (I)	conventional	FI	Sangiovese		StTus5
PI87	Greve in Chianti (II)	organic	FI	Sangiovese		StTus5
PI88	Greve in Chianti (II)	organic	FI	Sangiovese		StTus3

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518 **Table 1.** BNp genotypes, based on *vmp1* and *stamp* genes, identified in Tuscany on *Vitis vinifera* L.
 519 cv. Chardonnay and Sangiovese (part II)

BNp strain	Vineyard	Management	District	Cultivar	seq. var.	
					<i>vmp1</i>	<i>stamp</i>
48	Montecarlo (I)	conventional	LU	Chardonnay		StTus4
49	Montecarlo (I)	conventional	LU	Chardonnay	VmTus3	StTus3
50	Montecarlo (I)	conventional	LU	Chardonnay	VmTus4	StTus1
52	Montecarlo (I)	conventional	LU	Chardonnay	VmTus3	StTus1
53	Montecarlo (I)	conventional	LU	Chardonnay	VmTus3	StTus3
54	Montecarlo (II)	conventional	LU	Chardonnay		StTus4
201	Montecarlo (II)	conventional	LU	Chardonnay	VmTus5	StTus1
203	Montecarlo (II)	conventional	LU	Chardonnay		StTus1
258	Montecarlo (II)	conventional	LU	Chardonnay	VmTus3	StTus1
87	Massa	organic	MS	Sangiovese		
90	Massa	organic	MS	Chardonnay		StTus1
85	Massa	organic	MS	Sangiovese	VmTus5	StTus5
86	Massa	organic	MS	Sangiovese		
209	Licciana Nardi	organic	MS	Chardonnay	VmTus4	StTus4
11	Mulazzo	organic	MS	Chardonnay		
31	Carrara	organic	MS	Sangiovese	VmTus3	StTus3
106	San Gimignano	conventional	SI	Chardonnay		StTus3
108	San Gimignano	conventional	SI	Chardonnay	VmTus3	StTus3
109	San Gimignano	conventional	SI	Chardonnay	VmTus3	StTus3
110	San Gimignano	conventional	SI	Chardonnay		StTus3
PI21	Gaiole in Chianti (I)	conventional	SI	Chardonnay		StTus5
PI22	Gaiole in Chianti (I)	conventional	SI	Chardonnay	VmTus6	StTus3
PI24	Gaiole in Chianti (I)	conventional	SI	Chardonnay	VmTus6	StTus3
PI25	Gaiole in Chianti (I)	conventional	SI	Chardonnay		StTus2
PI11	Gaiole in Chianti (II)	conventional	SI	Chardonnay		StTus1
PI12	Gaiole in Chianti (II)	conventional	SI	Chardonnay		StTus3
PI14	Gaiole in Chianti (II)	conventional	SI	Chardonnay		StTus3
PI16	Gaiole in Chianti (II)	conventional	SI	Sangiovese		StTus3
PI17	Gaiole in Chianti (II)	conventional	SI	Sangiovese		StTus5
PI20	Gaiole in Chianti (II)	conventional	SI	Sangiovese		StTus3
PI68	Colle Val d'Elsa	conventional	SI	Chardonnay		StTus3
PI69	Colle Val d'Elsa	conventional	SI	Chardonnay		
PI61	Colle Val d'Elsa	conventional	SI	Sangiovese		
212	Montepulciano	conventional	SI	Sangiovese		StTus3
294	Montepulciano	conventional	SI	Sangiovese		StTus1
295	Montepulciano	conventional	SI	Sangiovese		StTus3
PI6	Montepulciano	conventional	SI	Sangiovese		StTus1
PI9	Montepulciano	conventional	SI	Sangiovese		StTus1
PI7	Montepulciano	conventional	SI	Sangiovese	VmTus6	StTus1

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3 521 **Table 2.** *vmp1* genetic variants of BNp strains identified in Tuscany in 2016, their prevalence,
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5 522 representative strains, TYPH10F/TYPH10R amplicon size, and sequence accession numbers
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7 523 deposited in NCBI GenBank.
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Variant	No. of strains	Representative strain	Size (nt)	Accession No.
VmTus1 (Vm39)	3	146	1296	MG874657
VmTus2 (Vm41)	3	190	1302	MG874658
VmTus3 (Vm43)	18	Pi77	1290	MG874659
VmTus4 (Vm88)	2	209	1266	MG874660
VmTus5 (Vm89)	2	85	1302	MG874661
VmTus6 (Vm90)	4	Pi43	1293	MG874662
VmTus7 (Vm91)	1	192	1296	MG874663
VmTus8 (Vm92)	1	186	1299	MG874664

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3 541 **Table 3.** *stamp* genetic variants of BNp strains identified in Tuscany in 2016, their prevalence,
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5 542 representative strains, STAMPF1/STAMPR1 amplicon size, and sequence accession numbers
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7 543 deposited in NCBI GenBank.
8

Variant	Num. Of strains	Representative strain	Size (nt)	Accession
StTus1 (St5)	22	146	474	MG874665
StTus2 (St9)	1	Pi25	495	MG874666
StTus3 (St10)	29	Pi77	474	MG874667
StTus4 (St11)	3	209	489	MG874668
StTus5 (St18)	8	85	477	MG874669

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Table 4. *vmp1/stamp* BNP genotypes identified in Tuscany in 2016, their prevalence and grouping in phylogenetic clusters

BNP genotype	No. BNP strains	<i>vmp1/stamp</i> cluster
VmTus1/StTus1 [Vm39/St5]	3	4
VmTus2/StTus1 [Vm41/St5]	1	1
VmTus2/StTus3 [Vm41/St10]	1	1
VmTus2/StTus5 [Vm41/St18]	1	1
VmTus3/StTus1 [Vm43/St5]	4	3
VmTus3/StTus3 [Vm43/St10]	11	3
VmTus4/StTus1 [Vm88/St5]	1	2
VmTus4/StTus4 [Vm88/St11]	1	2
VmTus5/StTus1 [Vm89/St5]	1	2
VmTus5/StTus5 [Vm89/St18]	1	2
VmTus6/StTus1 [Vm90/St5]	2	5
VmTus6/StTus3 [Vm90/St10]	2	5
VmTus7/StTus1 [Vm91/St5]	1	4
VmTus8/StTus3 [Vm92/St10]	1	3

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3 581 **FIGURE LEGENDS**

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5 582 **Pierro *et al.* [Prevalence of unusual '*Ca. P. solani*' strain in Tuscan grapevines]**

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9 584 **Figure 1.** Unrooted phylogenetic tree inferred from *vmp1* gene nucleotide sequences of BNp strains
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11 585 representative of *vmp1* sequence variants previously described (Table S1) and identified in this
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13 586 study (Table 2); minimum evolution analysis was performed using the neighbor-joining method and
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15 587 bootstrap replicated 1,000 times. Names of strains are reported on the image. GenBank accession
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17 588 number of each sequence is given in parenthesis; gene sequences obtained in the present study are
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19 589 indicated in bold. Clusters are shown as delimited by parentheses. Acronyms within clusters
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21 590 indicated phytoplasma hosts and origin. Hosts: Car, *Convolvulus arvensis*; Ho, *Hyalesthes*
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23 591 *obsoletus*; Lv, *Linaria vulgaris*; Sl, *Solanum lycopersicum*; St, *Solanum tuberosum*; Ud, *Urtica*
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25 592 *dioica*; Vv, *Vitis vinifera*. Origin: AU, Austria; B&H, Bosnia & Herzegovina; FR, France; GER,
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27 593 Germany; IT, Italy; MA, Macedonia; MONT, Montenegro; SLO, Slovenia.

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31 594 **Figure 2.** Unrooted phylogenetic tree inferred from *stamp* gene nucleotide sequences of BNp
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33 595 strains representative of *stamp* sequence variants previously described (Table S2) and identified in
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35 596 this study (Table 3); minimum evolution analysis was performed using the neighbor-joining method
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37 597 and bootstrap replicated 1,000 times. Names of strains are reported on the image. GenBank
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39 598 accession number of each sequence is given in parenthesis; gene sequences obtained in the present
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41 599 study are indicated in bold. Clusters are shown as delimited by parentheses. Acronyms within
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43 600 clusters indicated phytoplasma hosts and origin. Hosts: *Apium graveolens*, Ag; Ar, *Anaceratagallia*
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45 601 *ribauti*; Can, *Capsicum annuum*; Car, *Convolvulus arvensis*; Ho, *Hyalesthes obsoletus*; La,
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47 602 *Lavandula angustifolia*; *Phaseolus vulgaris*, Pv; Rp, *Reptalus panzeri*; Rq, *R. quinquecostatus*; Sl,
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49 603 *Solanum lycopersicum*; St, *Solanum tuberosum*; Ud, *Urtica dioica*; Va-c, *Vitex agnus-castus*; Vv;
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51 604 *Vitis vinifera*; Zm, *Zea mays*. Origin: AU, Austria; B&H, Bosnia & Herzegovina; BU, Bulgaria;
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53 605 CR, Croatia; FR, France; GEO, Georgia; GER, Germany; GR, Greece; IT, Italy; MA, Macedonia;
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55 606 MON, Montenegro; SER, Serbia; SLO, Slovenia.

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3 607 **Figure 3.** Unrooted phylogenetic tree inferred from concatenated nucleotide sequences of *vmp1* and
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5 608 *stamp* genes of BNp strains representative of *vmp1/stamp* types previously described (Table S3) and
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7 609 identified in this study (Table 1); minimum evolution analysis was performed using the neighbor-
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9 610 joining method and bootstrap replicated 1,000 times. Names of strains are reported on the image.
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11 611 GenBank accession number of each sequence is given in parenthesis; gene sequences obtained in
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13 612 the present study are indicated in bold. Clusters are shown as delimited by parentheses. Acronyms
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15 613 within clusters indicated phytoplasma hosts and origin. Hosts: Car, *Convolvulus arvensis*; Ho,
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17 614 *Hyalesthes obsoletus*; Sl, *Solanum lycopersicum*; St, *Solanum tuberosum*; Ud, *Urtica dioica*; Vv,
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19 615 *Vitis vinifera*. Origin: AU, Austria; B&H, Bosnia & Herzegovina; FR, France; GEO, Georgia; GER,
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21 616 Germany; IT, Italy; MA, Macedonia; SLO, Slovenia.

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24 617 **Figure 4.** Distribution of BNp strains, grouped in *vmp1/stamp* clusters, in north-western (N-W) and
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26 618 central-eastern (C-E) Tuscany. Number of *vmp1/stamp* clusters is showed inside the coloured
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28 619 rectangles within the graphic. Chi square test analyses showed statistically significant differences (p
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30 = 0.006) between N-W and C-E Tuscany.
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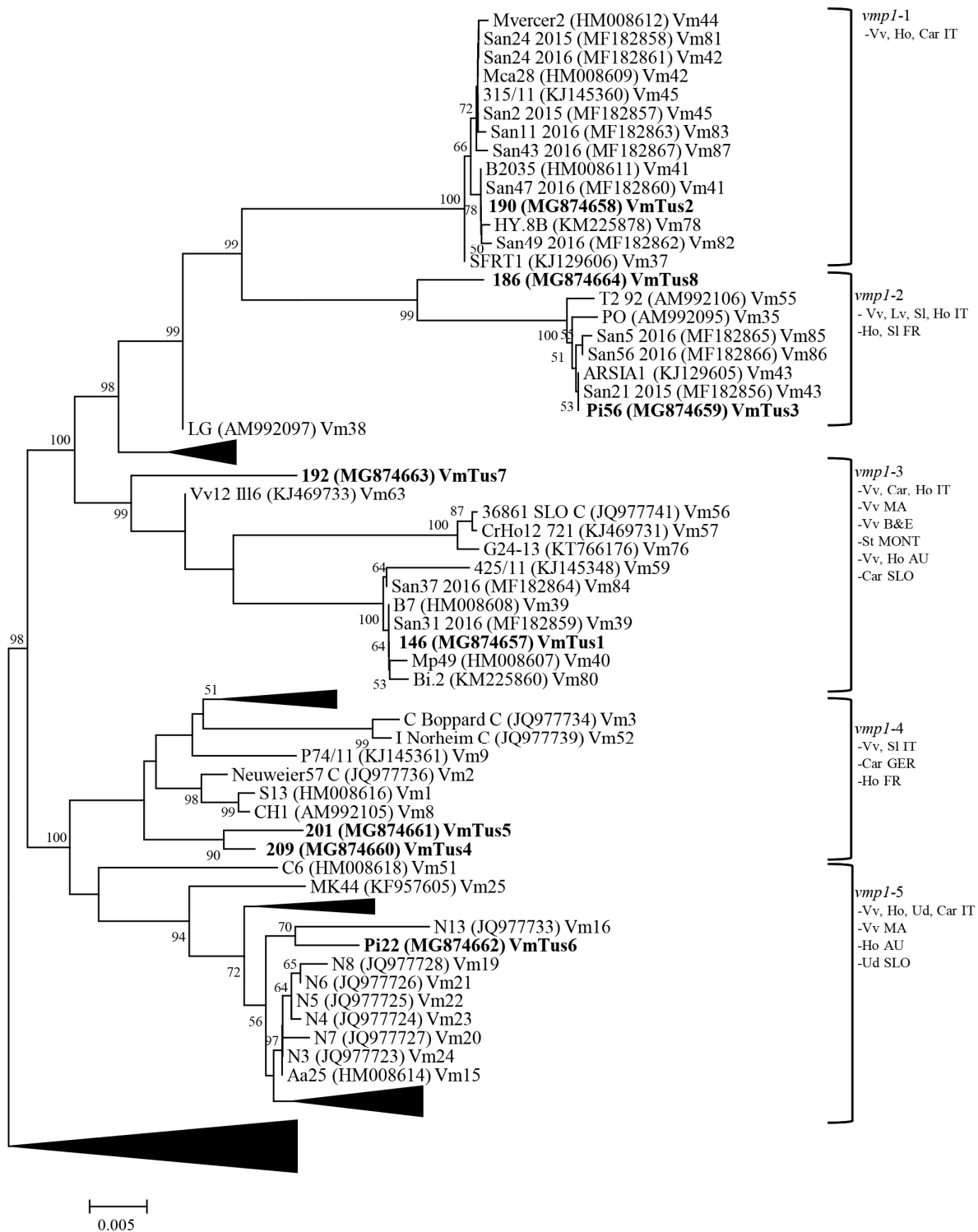
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Figure 1.



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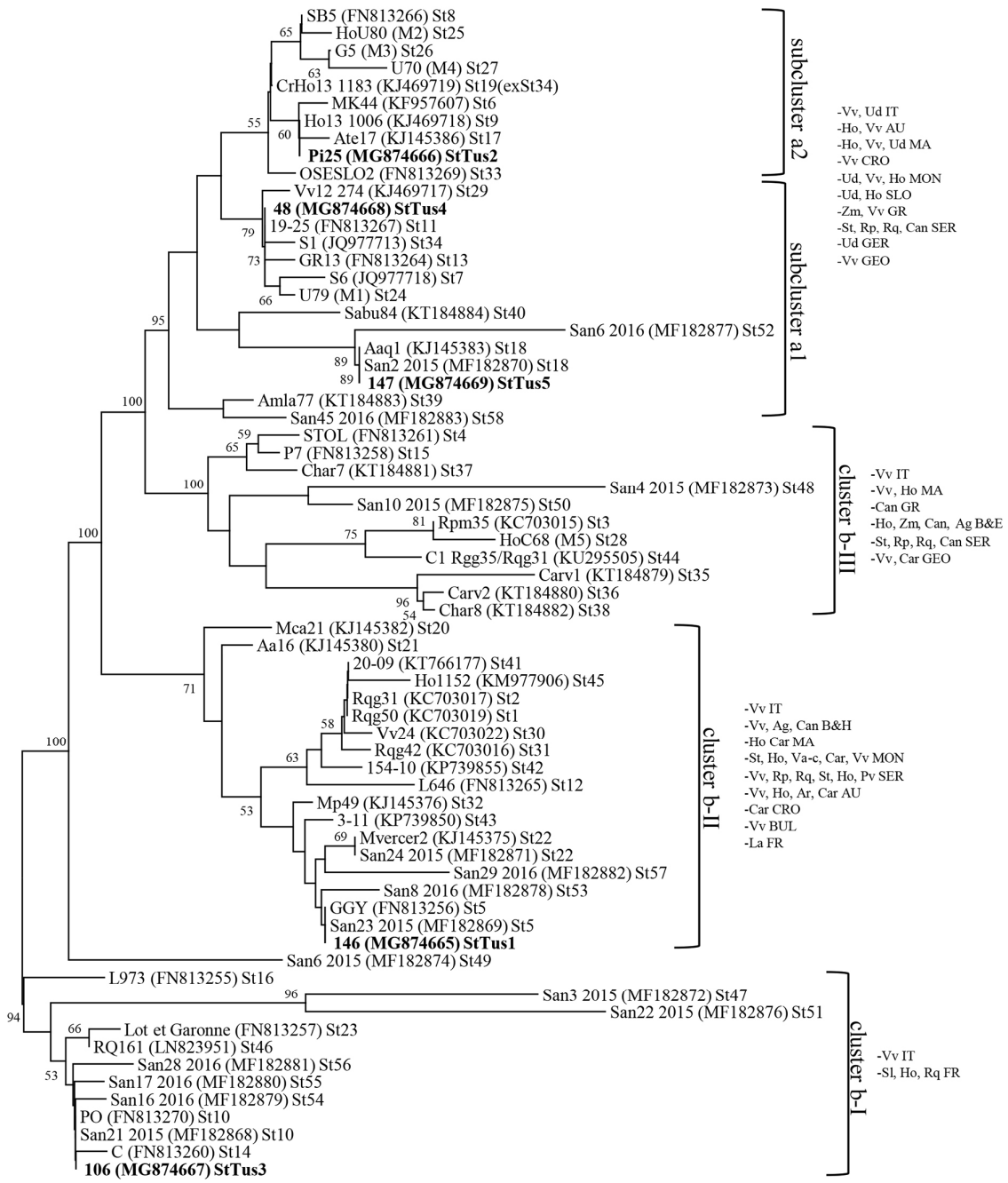
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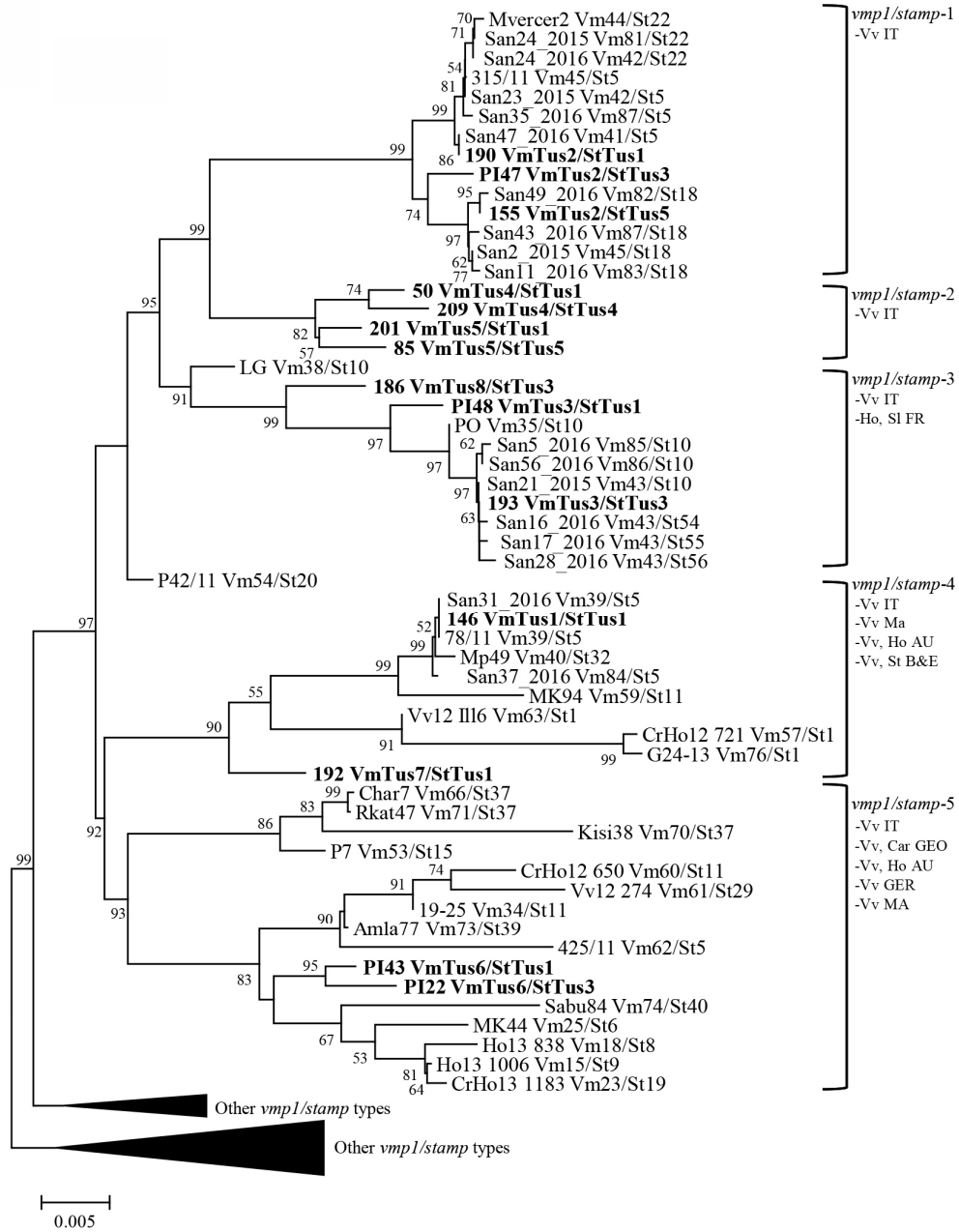
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Figure 2.



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Figure 3.



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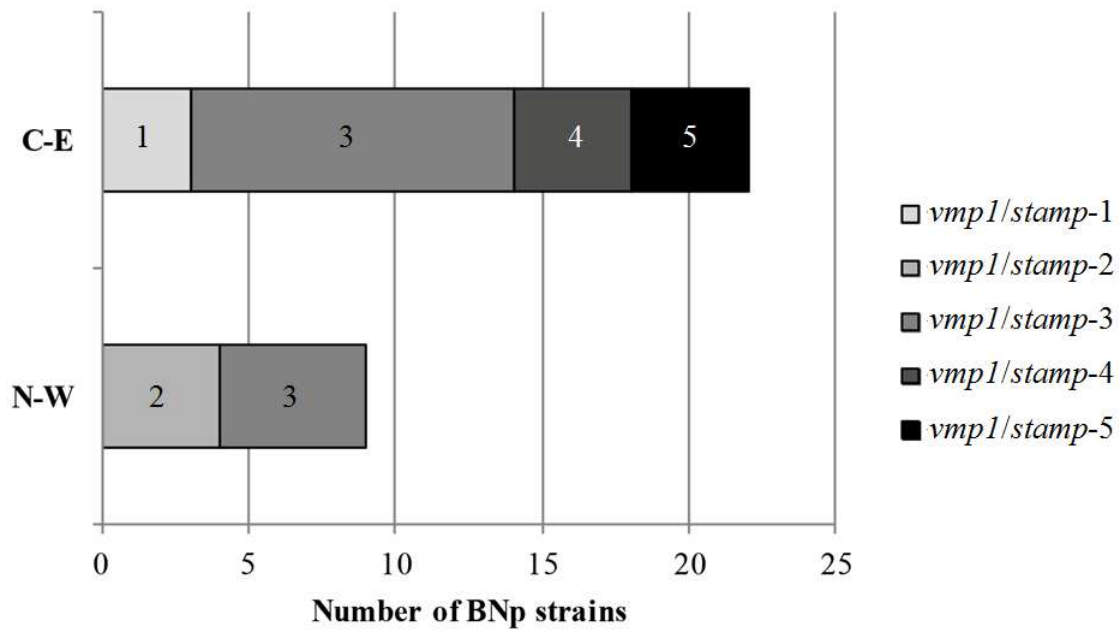
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Figure 4.



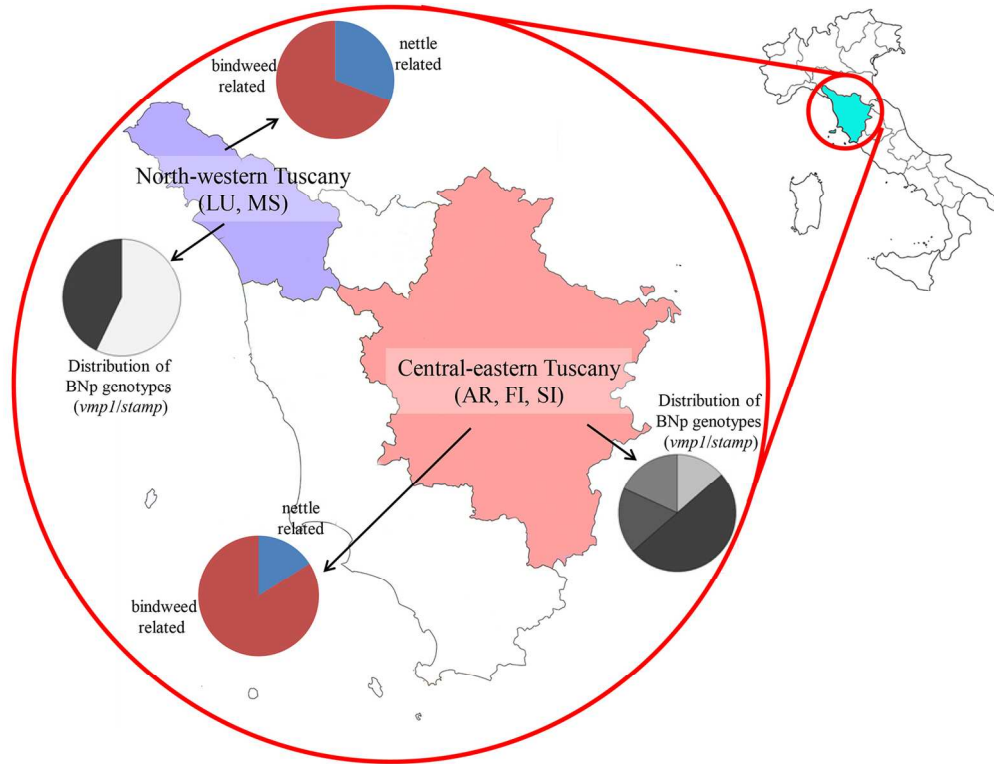
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Graphical abstract

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