New insights on “bois noir” epidemiology in the Chianti Classico area (Tuscany, central Italy)

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Running Title: New insights on bois noir epidemiology

SUMMARY

Bois noir (BN), a disease of the grapevine yellows complex associated with '*Candidatus* Phytoplasma solani' (*Ca*Psol), represents a great threat in all wine growing areas worldwide. Several studies revealed that BN epidemiology is extremely complex, including multiple insect vectors and plant hosts. In the present work, a survey on putative *Ca*Psol insect vectors along with molecular typing of *Ca*Psol strains was conducted ~~to in-depth investigate the BN epidemiology~~ in a BN-affected vineyard in ~~Greve in Chianti (~~the Chianti Classico area, ~~the~~ Tuscany region~~), where the role of *Reptalus artemisiae* in transmitting *Ca*Psol to grapevine was previously hypothesized~~. No *Hyalesthes obsoletus* specimens were captured, while *Reptalus~~.~~ artemisiae* and *Neoaliturus~~.~~ fenestratus* were found largely prevalent. Real-time PCR assay detected *Ca*Psol in 41% and 37.5% of *N. fenestratus* and *R. artemisiae* specimens, respectively. Molecular typing evidenced that *R. artemisiae* and *N. fenestratus* are infected by *Ca*Psol strains carrying mainly the *stamp* sequence variant St10 and St5, respectively. Other insects ~~(*Philaenus spumarius, Dictyophara europaea, Psammotettix* spp.)~~, reported as *Ca*Psol vectors to grapevine in North Italy and poorly present in ~~Greve in Chianti~~the examined vineyard, was found infected by *Ca*Psol strains carrying mainly the *stamp* sequence variant St5. In a recent study, these strains were found as the most abundant in grapevines (St5 and St10) and weeds (St10) in the ~~examined vineyard~~Chianti Classico area. Such results were reinforced by nucleotide sequence analyses of *secY* gene. Based on this and previous evidence, reporting the *Ca*Psol vectoring activity of both *R. artemisiae* and *N. fenestratus*, it is reasonable to suggest ~~that two main~~the role of such insects ~~cycles can be involved~~ in BN epidemiology in the Chianti Classico area. ~~: (i) grapevine – *R. artemisiae* – weeds for *Ca*Psol strains carrying the *stamp* variant St10; (ii) grapevine – *N. fenestratus* (plus other insects with a minor role) for *Ca*Psol strains carrying the *stamp* variant St5.~~ Thus, ~~F~~further studies are necessary to prove the vectoring activity of *Ca*Psol by *R. artemisiae* and *N. fenestratus* to grapevine and understand their ecological and epidemiological role in the vineyard agroecosystem, including *Ca*Psol source plants (insect host plants).~~, investigate the role of weeds in~~

~~*Ca*Psol transmission routes to grapevine, clarify if grapevine can act as reservoir plant for *Ca*Psol acquisition for these putative vectors~~.

Keywords: phytoplasmas; grapevine; insect vectors; weeds; *stamp*; *secY*

1 INTRODUCTION

Bois noir (BN), a disease of the grapevine yellows (GY) complex, represents a great threat in all major vine-growing areas in Europe, Mediterranean basin, Chile, South Africa, Middle East, and China (Bertaccini et al., 2018; Pierro et al., 2019). BN is associated with '*Candidatus* Phytoplasma solani' (*Ca*Psol), taxonomic subgroup 16SrXII-A (Quaglino et al., 2013). *Ca*Psol strains are mainly transmitted to grapevine by the insect vector *Hyalesthes obsoletus* Signoret, a polyphagous cixiid living preferentially on *Urtica dioica* L. (nettle), *Convolvulus arvensis* L. (bindweed), *Artemisia vulgaris* L., *Vitex agnus-castus* L., and *Crepis foetida* L., and erratically feeding on grapevine (Langer and Maixner, 2004; Sharon et al., 2005; Kosovac et al., 2019). In the last years, the utilization of variable (*secY*) and hyper-variable genes (*stamp*, *vmp1*) allowed more in-depth characterization of *Ca*Psol strain populations unveiling that alternative insect vectors and additional herbaceous plant hosts can play a role in the spreading of BN (Orenstein et al., 2003; Battle et al., 2008; Cvrković et al., 2014; Jakovljević et al., 2020; Mitrovic et al., 2019; Quaglino et al., 2019).

Interestingly, recent studies conducted in a BN-affected vineyard in Greve in Chianti (Chianti Classico area, Tuscany region) revealed the large prevalence of a *Ca*Psol strain carrying the *stamp* sequence variant St10, previously found mainly in Solanaceae hosts and in insect vectors in France and central Italy (Cimerman et al., 2009; Murolo & Romanazzi, 2015; Landi et al., 2015; Chuche et al., 2016), but never in grapevines outside of Tuscany (Pierro et al., 2018a, 2019). Epidemiological investigation carried out in the same vineyard, where *H. obsoletus* was not caught, showed the massive occurrence of *Reptalus artemisiae* (Becker), formerly know as *Reptalus quinquecostatus* (Duf.) (Emeljanov, 2020). *R. artemisiae* specimens were found infected by *Ca*Psol strains carrying

exclusively the *stamp* sequence variant St10, identified as prevalent also in grapevines and in most weeds (reservoir plants) for three consecutive years, suggesting the existence of a new BN epidemiological pattern related to these *Ca*Psol strains in which *R. artemisiae* can play a pivotal role (Pierro et al., 2020). Furthermore, the identification in grapevines and weeds of *Ca*Psol strains harboring *stamp* and *secY* sequence variants different from those detected in *R. artemisiae* can suggest the presence of further insect vectors involved in alternative BN epidemiological patterns in the studied vineyard (Pierro et al., 2020).

In the present work, a survey on putative *Ca*Psol insect vectors along with molecular typing of *Ca*Psol strains was conducted to improve the knowledge on alternative BN epidemiological patterns in Chianti Classico area.

2 MATERIALS AND METHODS

2.1 Insect survey

Survey on putative *Ca*Psol insect vectors was conducted in Greve in Chianti (Chianti Classico area, Florence district) inside an organic Sangiovese vineyard planted in 1997, with a density of 2.3 m between the rows and 0.8 m on the row. Ground cover was typical of the local area (Pierro et al., 2018). In previous studies carried out in this vineyard, *Ca*Psol strains carrying the *stamp* gene sequence variant St10 was found prevalent in BN-affected grapevines and in the putative vector *R. artemisiae*. From June to August 2019, a total of 9 yellow sticky traps were placed inside (6 traps) and at the vineyard borders (3 traps). The traps were positioned at the grapevine canopy level, 60 cm away from the ground, and replaced every 2 weeks. The insect identification was based on stereomicroscope observation of phenotypic characters and male genitalia after their dissection and clarification in a 10% potassium hydroxide solution. Specimens of putative insect vectors (phloem feeders) were kept at -20°C in ethanol 90% until DNA extraction.

2.2 DNA extraction and phytoplasma detection

Insect DNA was extracted from individual specimens with cetyltrimethylammonium bromide (CTAB) 2.5%, as described by Marzachì et al. (1998) with some modification. Briefly, each insect specimen was macerated using sterile sand in tube contain CTAB solution. The suspension was incubated for 20 min at 65°C, then extracted with chloroform-isoamyl alcohol (24:1). DNA was precipitated by adding 1 volume of cold isopropanol, recovered by centrifugation, washed with 70% ethanol and dried *in vacuo*. Obtained Pellets were dissolved in 50 µL of sterile water and stored in at -20°C until their use.

Detection of *Ca*Psol and phytoplasmas associated with Flavescence dorée (FD, taxonomic group 16SrV), and Aster Yellows (AY, taxonomic group 16SrI) was executed amplifying 16S rRNA gene through TaqMan assay (Angelini et al., 2007), using the Rotor-Gene Q (Qiagen, Germany). DNA extracted from the leaf veins of a healthy Sangiovese grapevine plant, maintained in the greenhouse of the Department of Agriculture, Food and Environment (University of Pisa, Italy), and reaction mixture devoid of DNA were used as negative controls.

2.3 Molecular typing and phylogenetic analyses of *Ca*Psol strains

*Ca*Psol strains, detected in the analyzed insect specimens, were characterized by nucleotide sequence analyses of the genes *stamp* and *secY*.

Stamp gene amplification was conducted by nested PCR using the primer pair *Stamp*F/*Stamp*R0 followed by *Stamp*F1/*Stamp*R1; reaction mixtures and PCR parameters were as described by Fabre et al. (2011). *SecY* gene amplification was performed in nested PCR using the primer pair *Posec*F1/*Posec*R1 followed by *Posec*F3/*Posec*R3; reaction mixtures and PCR parameters were as described by Fialovà et al. (2009). PCRs, carried out in the automated thermal cycler C1000 Cycler Touch (Bio-Rad), included the same controls described above for TaqMan assay. Amplification of the target genes was verified through electrophoresis on 1% agarose gel in Tris-borate-EDTA buffer and visualized under UV transilluminator.

Nested PCR amplicons of *stamp* and *secY* genes, representative of *Ca*Psol strains detected in insects, were sequenced in both strands (Sanger methods, 5x coverage per base position) by a commercial service (Eurofins Genomics, Germany). Nucleotide sequences were assembled by the Contig Assembling Program, trimmed to the annealing sites of the nested PCR primer pairs, aligned by ClustalW Multiple Alignment, and analyzed by Sequence Identity Matrix in the software BioEdit version 7.2.6 (Hall, 1999). Unique *stamp* and *secY* nucleotide sequences, identified in this study, were attributed to sequence variants (100% sequence identity) by their comparison with sequences previously deposited in GenBank and listed in reference Datasets previously published (Table S1, S2) (Pierro et al., 2018, 2019). Representative *stamp* and *secY* nucleotide sequences, identified in *Ca*Psol strains examined in the present study, were submitted to NCBI GenBank.

Representative *stamp* and *secY* sequence variants, identified in this and previous studies, were aligned for generating unrooted phylogenetic trees by Neighbour-Joining method performed using the Jukes–Cantor model and bootstrap replicated 1,000 times in the MEGA X software (Kumar et al., 2018).

3 RESULTS

3.1 *Ca*Psol detection in collected putative insect vectors

During the field survey carried out in the examined vineyard from June to August 2019, 179 *Auchenorrhyncha* adult insects were collected and distinguished, based on stereomicroscope analyses, in 8 taxonomic groups defined at species (5) and genus (3) level. Such insects belong to the family Cicadellidae (97 specimens), Cixiidae (56), Aphrophoridae (13), and Dictiopharidae (13) (Table 1). The most abundant insect species were *Neoaliturus fenestratus* (Herrich-Schaffer) (78 specimens; 43.6%), *R. artemisiae* (56 specimens; 31.3%), and *Dictyophara europaea* (Linnaeus) (7.3%) (Table 1).

TaqMan assay detected *Ca*Psol in 60 out of 179 collected insect specimens. In detail, *Ca*Psol was detected in five insect taxa with different infection rate: 41% in *N. fenestratus*, 37.5% in *R. artemisiae*, 30% in *Philaenus spumarius* Linnaeus, 20% in *Psammotettix* sp., and 15% in *D. europaea*. No phytoplasmas were found in *Neophilaenus* sp., *Macrosteles* sp., and *Zygina rhamni* (Ferrari) (Table 1). Phytoplasmas associated with AY and FD were not identified in any insect. Real-time PCR analysis gave no amplification in negative controls (healthy grapevine control plant and reaction mixtures devoid of nucleic acids). The 60 *Ca*Psol strains detected in insect specimens were furtherly characterized by nucleotide sequence analyses of *stamp* and *secY* genes.

3.2 *Ca*Psol strain molecular typing

stamp gene amplicons of the expected size (around 600 nt) were obtained in 50 out of 60 insect specimens infected by *Ca*Psol. Nucleotide sequence alignments and comparison with the updated dataset of *stamp* sequence variants (Table S1) allowed the identification of four distinct *stamp* sequence variants in *Ca*Psol strains infecting insects. The two prevalent variants, carried by 37 out of 50 *Ca*Psol strains, shared 100% of sequence homology with variants St5 (22 strains) and St10 (15 strains); the remaining two *stamp* variants were firstly reported in this study and named St60 and St61 (Table 2; Table S3). These new *stamp* variants shared the best sequence identity with previously described variants: St60 showed 98.73% (6 single nucleotide polymorphisms, SNP) of sequence identity compared with St10, while St61 98.73% (5 SNPs) of sequence identity compared with St5. Prevalence of *Ca*Psol strains carrying distinct *stamp* variants was different in phytoplasma-infected insects. *N. fenestratus* was found infected by *Ca*Psol strains characterized by the *stamp* variants St5 (18 specimens out of 23), St61 (3), and St60 (2); *R. artemisiae* by *Ca*Psol strains characterized by the *stamp* variants St10 (15 out of 20) and St60 (5); *P. spumarius* by *Ca*Psol strains characterized by the *stamp* variant St61 (3); *Psammotettix* sp. (2 specimens) and *D. europaea* (2) by strains carrying the variant St5 (Table 3; Table S3).

SecY gene amplicons of the expected size (around 950 nt) were obtained in 31 out of 60 insect specimens infected by *Ca*Psol. Nucleotide sequence alignments and comparison with the updated dataset of *secY* sequence variants (Table S2) allowed the identification of five distinct *secY* sequence variants in *Ca*Psol strains infecting insects. Four *secY* variants, identified in 14 out of 31 *Ca*Psol strains, shared 100% of sequence homology with the variants SecY1 (4 strains), ecY6 (4 strains), SecY9 (one strain), and SecY33 (5 strains). The remnant sequence variant (named SecY35), firstly reported in this study and identified in 17 *Ca*Psol strains, shared 99.8% (3 SNPs) of sequence homology with the variant secY6. Prevalence of *Ca*Psol strains carrying distinct *secY* variants was different in phytoplasma-infected insects. *N. fenestratus* was found infected by *Ca*Psol strains characterized by the *secY* variants SecY35 (8 specimens out of 11), SecY33 (2), and SecY9 (1); *R. artemisiae* by *Ca*Psol strains characterized by the *secY* variants SecY35 (9 out of 16), SecY6 (4), and SecY33 (3); *P. spumarius* (2 strains) and *D. europaea* (2 strains) by *Ca*Psol strains characterized by the SecY1 (Table 3).

3.3 Phylogenetic analyses

Two main bindweed-related sub-clusters were identified in the phylogenetic tree generated by the analysis of the *stamp* sequence variants found in the present study and those reported in the dataset (Table S1). St5 and St60 grouped in the cluster b-II, along with other *Ca*Psol strains previously identified in different European countries and Balkans. St10 and St61 grouped in the cluster b-I, along with other *Ca*Psol strains previously identified in Italy and France (Figure 1).

Three main clusters were identified in the phylogenetic tree generated by the analysis of the *secY* sequence variants identified in this study and those reported in the dataset (Table S2). *Ca*Psol strains carrying the SecY1 variant grouped within the cluster *SecY*-1; the variants SecY9 and SecY33 in the cluster *SecY*-2; the variants SecY6 and SecY35 grouped within the cluster *SecY*-3 (Figure 2). No specific variant groupings were correlated with geographical origin.

4 DISCUSSION

In Italy and other European countries, *Ca*Psol is transmitted to grapevine mainly by the vector *H. obsoletus* (Bertaccini, 2018). Data obtained from the survey on BN putative vectors, carried out in 2019 in the examined vineyard in Greve in Chianti, showed that no *H. obsoletus* specimens were captured, confirming the results obtained in previous study (Pierro et al., 2020). Considering that GY incidence on the studied vineyard has been increasing through the last years and FD phytoplasmas were never identified in symptomatic grapevines (Pierro et al., 2018, 2018a, 2019, 2020), it can reinforce the hypothesis that other epidemiological patterns could be involved in *Ca*Psol transmission to grapevines in the examined vineyard. *R. artemisiae*, formerly known as *R. quinquecostatus* (Emeljanov, 2020), and *N. fenestratus* were found largely prevalent among captured putative vectors. Moreover, *P. spumarius*, *D. europaea*, and *Psammotettix* sp., recently reported as alternative vectors of *Ca*Psol to grapevine in northern Italy (Quaglino et al., 2019), were found in the examined vineyard.

In the last ten years, a larger genetic diversity among *Ca*Psol strains was described by molecular characterization of hypervariable genes (i.e., s*ecY*, *vmp1*, and *stamp*) (Murolo & Romanazzi, 2015). Interestingly, studies focused on *stamp* gene molecular markers improved the knowledge on *Ca*Psol strain population structure and dynamics (Pierro et al., 2018), revealing the phytoplasma transmission ways in vineyard agro-ecosystems (Kosovac et al., 2016). In particular, the specific phytoplasma-vector recognition mechanism involves the binding of insect cytoskeleton proteins with the antigenic membrane protein encoded by the *Ca*Psol *stamp* gene. Thus, *stamp*-based molecular typing of *Ca*Psol strains has been employed to identify its insect vectors and transmission routes in previous studies (Chuche et al., 2016; Kosovac et al., 2016; Quaglino et al., 2019).

Previous studies, conducted in the examined vineyard, highlighted that bindweed-related *Ca*Psol strains carrying the *stamp* sequence variants St5, St10, and St18 were largely prevalent in affected grapevines throughout years (Pierro et al., 2018, 2019, 2020). In comparison with previous study (Pierro et al., 2020), *Ca*Psol molecular detection and typing confirmed the high infection rate

of *R. artemisiae* specimens carrying bindweed-related phytoplasma strains mainly characterized by the *stamp* variant St10 (phylogenetic cluster b-I). Additionally, *Ca*Psol strains found in *R. artemisiae* carried the *secY* sequence variant SecY6, SecY33, and SecY35 (similar to SecY6), previously identified in affected grapevines (Pierro et al., 2020). This result reinforced the hypothesis that a new BN epidemiological pattern, involving grapevine, *R. artemisiae*, and weeds, can be present in the Chianti Classico area.

Interestingly, *Ca*Psol molecular detection and typing revealed the high infection rate of *N. fenestratus* specimens carrying bindweed-related phytoplasma strains mainly characterized by the *stamp* variant St5 (phylogenetic cluster b-II) and *secY* variants SecY35 (similar to SecY6), SecY33, and SecY9, previously found in affected grapevines in Greve in Chianti (Pierro et al., 2020). *N. fenestratus* is a polyphagous insect, living preferentially on herbaceous plants, commonly reported in vineyard agroecosystems in Italy and Mediterranean countries (Bosco et al., 1997; Orenstein et al., 2003; Lessio et al., 2017). It was found as *Ca*Psol insect host in BN vineyards in diverse Italian regions (Landi et al., 2013; Conigliaro et al., 2020), and as *Ca*Psol insect vector to lettuce and carrot in Serbia (Mitrovic et al., 2019). Moreover, in a recent study, *N. fenestratus* was reported as natural vector of 16SrII phytoplasmas to hawkweed oxtongue (*Picris hieracioides*) affected by bushy stunt in south-eastern Serbia (Mitrovic et al., 2012). Moreover, *P. spumarius*, *D. europaea*, and *Psammotettix* sp., recently reported as alternative vector of *Ca*Psol to grapevine in North Italy (Quaglino et al., 2019) and not abundant in Greve in Chianti, were found infected by the same *Ca*Psol strains carrying the variant St5. Based on this evidence, it is reasonable to suggest that another epidemiological cycle, including grapevine and at least *N. fenestratus*, can be present in the examined vineyard for *Ca*Psol strains carrying the variant St5. The fact that such *Ca*Psol strain (St5) was not detected in weeds in Greve in Chianti (Pierro et al., 2020) could indicate that affected grapevines can be a reservoir plant for these putative vectors.

Based on the data obtained in this and previous study (Pierro et al., 2020), two main BN epidemiological patterns are proposed for bindweed-related *Ca*Psol strains in the Chianti Classico

247 area: (i) grapevine – *R. artememisiae* - weeds for strains carrying the *stamp* variant St10 (phylogenetic
248 cluster b-I); (ii) grapevine – *N. fenestratus* (minor role for *P. spumarius*, *D. europaea*, and
249 *Psammotettix* sp.) for strains carrying the variant St5 (phylogenetic cluster b-II).
250 Further studies are necessary to prove the vectoring activity of *Ca*Psol by *R. artemisiae* and
251 *N. fenestratus* to grapevine, investigate the role of weeds in *Ca*Psol transmission routes to grapevine,
252 clarify if grapevine can act as reservoir plant for *Ca*Psol acquisition for these putative vectors.
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361 **Table 1**. Identification of *Ca*Psol in insect specimens collected in the examined vineyard

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Family	**Species**	**No. of specimens**		
		CaPsol-infected / collected	**PCR *stamp***	**PCR *secY***
			positive / infected	**positive / infected**
Aphrophoridae	*Philaenus spumarius*	3/10	3/3	2/3
	Neophilaenus spp.	0/3	-	-
Cicadellidae	*Neoaliturus fenestratus*	32/78	23/32	16/32
	Psammotettix spp.	2/10	2/2	-
	Zygina ramnhi	0/8	-	-
	Macrosteles spp.	0/1	-	-
Cixiidae	*Reptalus artemisiae*	21/56	20/21	11/21
Dictiopharidae	*Dictyophara europaea*	2/13	2/2	2/2

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364 **Table 2**. *stamp* sequence variants carried by *Ca*Psol strains identified in insects

***stamp* sequence variant**	**No. of *Ca*Psol strains**	**Insect species**	**Acc. No.**
St5	18	*N. fenestratus*	MZ06536
St60	2	*N. fenestratus*	MZ065361
St61	3	*N. fenestratus*	MZ065360
St10	15	*R. artemisiae*	MZ065363
St60	5	*R. artemisiae*	MZ065361
St61	3	*P. spumarius*	MZ065360
St5	2	*Psammotettix* spp.	OK376608
St5	2	*D. europaea*	OK376607

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Table 3. *secY* sequence variants carried by *Ca*Psol strains identified in insects

***secY* sequence variant**	**No. of *Ca*Psol strains**	**Insect species**	**Acc. No.**
SecY33	2	*N. fenestratus*	OK376614
SecY35	8	*N. fenestratus*	OK376615
SecY9	1	*N. fenestratus*	OK376612
SecY33	3	*R. artemisiae*	OK376613
SecY35	9	*R. artemisiae*	OK376616
SecY6	4	*R. artemisiae*	OK376611
SecY1	2	*P. spumarius*	OK376610
SecY1	2	*D. europaea*	OK376609

Figure 1. Unrooted phylogenetic tree inferred from *stamp* gene nucleotide sequences of '*Ca*Psol strains representative of stamp sequence variants previously described (Table S1) and identified in the present study (Table 2); minimum evolution analysis was performed using the neighbour-joining method and bootstrap replicated 1,000 times. Names of strains are reported on the image. GenBank accession number of each sequence is given in parenthesis; gene sequences obtained in the present study are indicated by black circle. Clusters are shown as delimitated by parentheses. Acronyms within clusters indicated phytoplasma hosts and origin. Hosts: Ag, *Apium graveolens*; Ar, *Anaceratagallia ribauti*; Can, *Capsicum annuum*; Car, *Convolvulus arvensis*; Ho, *Hyalesthes obsoletus*; La, *Lavandula angustifolia*; Nf, *Neoaliturus fenestratus*; Ps, *Philaenus spumarius;* Pv, *Phaseolus vulgaris*; Rp, *Reptalus panzeri*; Rq, *R. quinquecostatus*; Sl, *Solanum lycopersicum*; St, *Solanum tuberosum*; Ud, *Urtica dioica*; Va-c, *Vitex agnus-castus*; Vv; *Vitis vinifera*; Zm, *Zea mays*. Origin: AU, Austria; B&H, Bosnia & Herzegovina; BU, Bulgaria; CR, Croatia; FR, France; GEO, Georgia; GER, Germany; GR, Greece; IT, Italy; MA, Republic of Macedonia; MON, Montenegro; SER, Serbia; SLO, Slovenia.

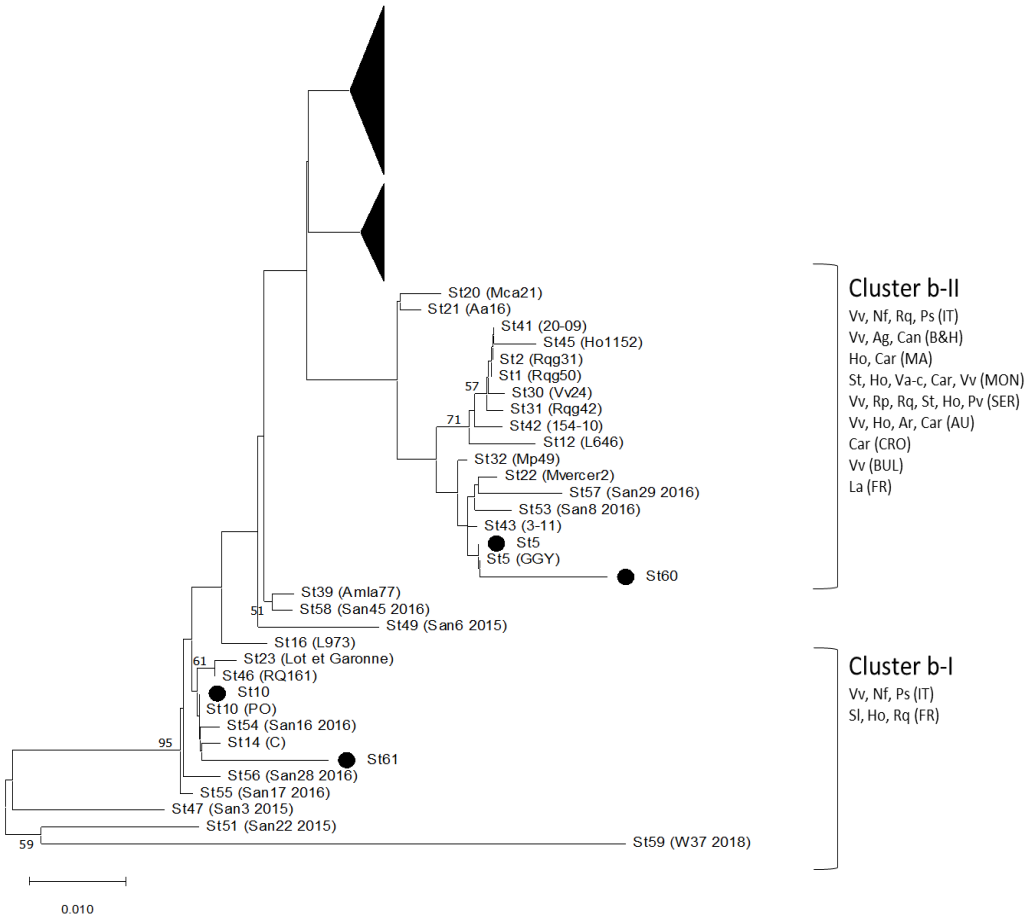

Figure 2. Unrooted phylogenetic tree inferred from *secY* gene nucleotide sequences of *Ca*Psol strains representative of *secY* sequence variants previously described (Table S2) and identified in this study (Table 2); minimum evolution analysis was performed using the neighbour-joining method and bootstrap replicated 1000 times. Names of strains are reported on the image and nucleotide sequences obtained in this study are in bold. Clusters are shown as delimitated by parentheses. Acronyms within clusters indicated phytoplasma hosts and origin. Hosts: Ca, *Convolvulus arvensis*; Cmin, *Centaurea minus*; Cros, *Catharanthus roseus*; Cv, *Clematis vitalba*; Ho, *Hyalesthes obsoletus*; La, *Lavandula angustifolia*; Mcha, *Matricaria chamomilla*; Nar, *Narcissus* sp.; Nf, *Neoaliturus fenestratus*; Phav, *Phaseulus vulgaris*; Phie, *Picris hieracioides*; Ps, *Philaenus spumarius*; Rno, *Reptalus noahi*; Rp, *Reptalus panzeri*; Rq, *Reptalus quinquecostatus*; Sl, *Solanum lycopersicum*; Sm, *Salvia miltiorrhiza*; Son, *Sonchus* sp.; Sscl, *Salvia sclarea*; St, *Solanum tuberosum*; Ud, *Urtica dioica*; Vitex, *Vitex agnus-castus*; Vv, *Vitis vinifera*; Zm, *Zea mays*. Origin: AU, Austria; AZER, Azerbaijan; CH, China; CR, Croatia; FR, France; GEO, Georgia; GER, Germany; GR, Greece; IRA, Iran; IT, Italy; LEB, Lebanon; MON, Montenegro; SER, Serbia; SLO, Slovenia; SWIT, Switzerland; YUGO, Yugoslavia.