Distribution and Genetic Diversity of *Xylella fastidiosa* subsp. *pauca* Associated with Olive Quick Syndrome Symptoms in Southeastern Brazil

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ABSTRACT

In Brazil, the host expansion of *Xylella fastidiosa* subsp. *pauca* was recently demonstrated with the report of diseased olive trees (*Olea europaea*), whose symptoms were associated with olive quick decline syndrome previously described in southern Italy. We employed both polymerase chain reaction-based techniques and culture medium isolation to investigate the geographic distribution of *X. fastidiosa* as well as the genetic signatures of 21 strains isolated from 11 olive orchards in both São Paulo and Minas Gerais States in Brazil. *X. fastidiosa* subsp. *pauca* was detected in 83% of the orchards examined in the region, and was positively diagnosed in 43.7% of all sampled plants with typical scorching symptoms. Of the 21 strains characterized with fast-evolving

microsatellite (single sequence repeat [SSR]) markers, 20 different multilocus microsatellite genotypes were observed with the overall allelic diversity of $H_{Nei} = 0.38$. Principal component analysis using the SSR markers clustered all strains, except for three, in one cluster demonstrating a limited range of genetic diversity. Multilocus sequence typing analysis showed the prevalence of a sequence type (ST16) in 75% of the samples; three other novel STs (84, 85, and 86), were detected, all belonging to the *X. fastidiosa* subsp. *pauca* cluster. These results show that genetically diverse strains of *X. fastidiosa* subsp. *pauca* are widely present in olive orchards in southeastern Brazil, which is consistent with the long history of this bacterium in that region.

The xylem-inhabiting bacterium *Xylella fastidiosa* is the etiological agent of some of the most important plant diseases that have recently emerged worldwide (Almeida and Nunney 2015). Diseases caused by *X. fastidiosa* exhibit a complex pathosystem; this pathogen is able to infect over 350 plant taxa but does not always cause disease symptoms (EFSA 2016). Furthermore, the bacterium plant-to-plant dispersion occurs naturally by hundreds of species of polyphagous xylem-sap feeding insects (Sicard et al. 2018). In addition, environmental conditions (Purcell 2013) and agricultural practices (Sicard et al. 2018) could affect the severity of the diseases caused by *X. fastidiosa*.

The species *X. fastidiosa* is divided into five clades (subspecies) based on DNA–DNA hybridization, internal transcribed spacer sequences, and, more recently, multilocus sequence typing (MLST) using the partial sequences of seven housekeeping genes (Schaad et al. 2004; Yuan et al. 2010) (<https://pubmlst.org/xfastidiosa/>). *X. fastidiosa* subsp. *fastidiosa*, *X. fastidiosa* subsp. *multiplex*, *X. fastidiosa* subsp. *sandyi*, and *X. fastidiosa* subsp. *morus* were first found in North America (Nunney et al. 2013; Schuenzel et al. 2005), although subsp. *multiplex* is also present in South America and subsp. *fastidiosa* is hypothesized to have originated from Central America (Coletta-Filho et al. 2017; Nunney et al. 2010). On the

other hand, *X. fastidiosa* subsp. *pauca* was identified as being restricted to South America, infecting citrus and coffee plants (Schuenzel et al. 2005). However, recent studies demonstrated that subsp. *pauca* has expanded to different regions and hosts in Europe and Central America (Nunney et al. 2014; Saponari et al. 2017).

The risks of introducing new *X. fastidiosa* strains in areas already experiencing epidemics of this pathogen include an increase in genetic diversity, the emergence of new diseases, and host range expansion (Sicard et al. 2018). Moreover, human-altered natural habitats could induce evolutionary changes in pathogens that would also drive the invasion of plant pests to novel hosts (Hufbauer et al. 2012). The unintentional introduction of *X. fastidiosa* subsp. *fastidiosa* by anthropogenic activities such as moving exotic plant species to a new geographic area, in combination with favorable environmental conditions, resulted in the Pierce’s disease of grapevine outbreak in Southern California, as discussed by Nunney et al. (2010). Recently, a new outbreak caused by *X. fastidiosa* subsp. *pauca* has affected olive trees in southern Italy. Evidence indicates that the introduction of infected plant material carrying a single *X. fastidiosa* genotype and favorable environmental conditions were sufficient for the emergence of the olive quick decline syndrome (OQDS) disease (Sicard et al. 2018).

The young olive oil industry in southeast Brazil occupies areas located at high altitudes such as the mountain chain known as “Serra da Mantiqueira”, with small orchards surrounded by native vegetation. This scenery could be favorable for the migration of *X. fastidiosa* from native vegetation to crops (Purcell and Saunders 1999). Although planting of olive crops is a recent agricultural activity, *X. fastidiosa* subsp. *pauca* has already been associated with olive plants sampled in Brazil (Coletta-Filho et al. 2016).

Because the Brazilian population of *X. fastidiosa* subsp. *pauca* is genetically diverse (Coletta-Filho et al. 2017), with favorable environmental conditions in olive orchards for pathogen spread, it is necessary to investigate the spatial distribution of these bacteria through the olive production areas in southeast Brazil and the

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genetic diversity of the strains present in plants with OQDS-like symptoms. Here, we present data on the genetic diversity and dispersion of *X. fastidiosa* in olive orchards in southeastern Brazil.

MATERIALS AND METHODS

Sampling. In total, 395 plants from olive orchards located in the southeast region of Brazil (São Paulo and Minas Gerais States) at different altitudes and with variable landscape vegetation were sampled for diagnosis of *X. fastidiosa* (Table 1). Plants with suspicious, OQDS-like symptoms as described by Saponari et al. (2017) were sampled. Branches (3 to 5 mm in diameter) containing suspicious symptomatic leaves (i.e., scorching symptoms) were sampled and transferred to the laboratory for diagnosis, bacterial isolation, and molecular typing of *X. fastidiosa*.

***X. fastidiosa* diagnoses and isolation.** The presence of *X. fastidiosa* in the samples was determined by polymerase chain reaction (PCR) using the RST 31/33 *X. fastidiosa*-specific primer set (Minsavage et al. 1994) with amplification conditions specified by the authors; approximately 100 ng of total DNA was used in a final volume of 20 µl per reaction. For DNA extraction, we used petiole and midribs of green leaves sampled from asymptomatic portions of symptomatic branches, using a cetyl trimethyl ammonium bromide (CTAB)-based protocol adapted from Murray and Thompson (1980). *X. fastidiosa* was tentatively isolated using the same portion of olive tissue used for the PCR-based diagnosis. First, the branches were superficially disinfested with 70% ethanol for 2 min, immersed in a 2% bleach solution for 2 min, and washed three times in sterilized water. After being dried on a sterile bench in a laminar flow hood, the branches were cut in the middle, the

TABLE 1. General information about *Xylella fastidiosa* subsp. *paucis* diagnosis and surveyed olive orchards in southeast Brazil

Origin (state, area) ^a	Latitude, longitude	Altitude (m)	Number of plants ^b	<i>X. fastidiosa</i> -positive plants ^c	Plants with <i>X. fastidiosa</i> isolation ^d	Landscape vegetation
Minas Gerais, A1	22°18'56.55", 45°22'30.49"	1,300	57	48 (84.2)	16	Araucaria forest
Minas Gerais, A2	22°18'51.67", 45°22'40.82"	1,310	22	16 (72.7)	4	Araucaria forest and stone fruits
Minas Gerais, A3	22°18'45.01", 45°22'23.40"	1,329	23	5 (21.7)	1	Araucaria forest
Minas Gerais, A4	22°18'45.01", 45°22'23.40"	1,318	33	19 (57.5)	4	Araucaria forest
Minas Gerais, A5	22°35'46.45", 45°55'25.88"	1,235	39	6 (15.3)	2	Araucaria and ombrophilous forests
Minas Gerais, A6	22°10'47.57", 44°36'17.71"	1,200	14	7 (50.0)	0	Araucaria and ombrophilous forests
Minas Gerais, A7	22°59'52.60", 44°40'27.31"	1,400	4	0 (0.0)	0	Araucaria and ombrophilous forests
Minas Gerais, A8	22°01'5.55", 44°39'32.90"	1,500	4	4 (100)	0	Araucaria and ombrophilous forests
Minas Gerais, A9	22°01'8.58", 44°37'23.17"	1,300	9	6 (66.6)	0	Araucaria and ombrophilous forests
Minas Gerais, A10	22°18'16.85", 45°24'32.87"	1,780	10	0 (0.0)	1	Araucaria and ombrophilous forests
Minas Gerais, A11	22°30'29.87", 44°18'7.09"	1,250	1	0 (0.0)	0	Ombrophilous forests
Minas Gerais, A12	21°57'31.16v, 46° 21'19.96"	1,380	12	7 (58.3)	0	Ombrophilous forests, grass
Minas Gerais, A13	21°1'54.44v, 46°35'3.77"	1,050	12	1 (8.3)	3	Araucaria and ombrophilous forests
Minas Gerais, A14	22°18'16.85", 44°58'34.03"	1,065	3	1 (33.3)	0	Small area of ombrophilous forests, grasses
Minas Gerais, A15	20°41'7.35", 43°28'9.34"	854	1	1 (100)	0	Ombrophilous forests
Minas Gerais, A16	19°40'20.63", 43°35'11.67"	987	1	0 (0.0)	0	Ombrophilous forests
Minas Gerais, A17	20°19'54.88", 43°45'19.93"	883	1	1 (100)	1	Ombrophilous forests, grasses
Minas Gerais, A18	22°35'8.84", 45°50'33.50"	1,329	6	0 (0.0)	3	Araucaria and ombrophilous forests
São Paulo, A1	22°42'10.71", 45°41'32.91"	1,602	40	16 (40.0)	4	Araucaria and ombrophilous forests
São Paulo, A2	23°45'37.30", 47°44'45.47"	690	60	18 (30.0)	3	Small area of tropical forests
São Paulo, A3	22°37'41.72", 44°53'55.70"	615	5	5 (100.0)	NR	Ombrophilous forests, grasses
São Paulo, A4	22°30'13.54", 46°36'45.39"	1,054	2	0 (0.0)	NR	Araucaria forest
São Paulo, A5	23°31'13.64", 46°56'11.21"	755	20	3 (15.0)	1	No vegetation, grasses
São Paulo, A6	23°16'22.23", 47°09'9.42"	883	16	3 (18.7)	2	Pine forest, eucalyptus, grasses
Overall	...	...	395	167 (43.7) ^e	45	...

^a Areas (orchards) in Minas Gerais and São Paulo States of Brazil used for sampling.

^b Total number of tested plants showing olive quick decline syndrome-like symptoms.

^c Number of *Xylella fastidiosa* polymerase chain reaction (PCR)-positive plants using the RST31/RST33 primers. The percentage is shown in parentheses.

^d Number of plants with successful *X. fastidiosa* isolation in buffered charcoal yeast extract (BCYE) agar medium. Plants with a *X. fastidiosa*-positive isolation but negative PCR amplification are shown in bold. NR = no tentative isolation.

^e Considering both positive PCR amplification and successful isolation of *X. fastidiosa*.

TABLE 2. Single sequence repeat (SSR)-based genetic diversity of *Xylella fastidiosa* strains ($n = 21$) isolated from olive trees in southeast Brazil

Locus	Genetic diversity of <i>X. fastidiosa</i> strains according to geographic origin ^a				
	All areas	MG areas A1 to A4	SP area A1	SP area A2	Other ^b
COSSR4	0.83 (9)	0.60 (5)	0.17 (2)	0.17 (2)	0.50 (3)
CSSR42	0.00 (1)	0.00 (1)	0.00 (1)	0.00 (1)	0.00 (1)
OSSR19	0.38 (6)	0.00 (1)	0.67 (4)	0.00 (1)	0.17 (2)
OSSR14	0.29 (3)	0.00 (1)	0.17 (2)	0.00 (1)	0.17 (2)
OSSR17	0.77 (8)	0.68 (5)	0.50 (3)	0.17 (2)	0.17 (2)
GSSR4	0.22 (4)	0.00 (1)	0.67 (4)	0.00 (1)	0.00 (1)
COSSR6	0.55 (5)	0.46 (4)	0.50 (3)	0.00 (1)	0.17 (2)
GSSR12	0.00 (1)	0.00 (1)	0.00 (1)	0.00 (1)	0.00 (1)
COSSR1	0.37 (5)	0.00 (1)	0.58 (3)	0.00 (1)	0.50 (3)
COSSR3	0.30 (4)	0.00 (1)	0.50 (3)	0.00 (1)	0.17 (2)
CSSR18	0.22 (4)	0.00 (1)	0.67 (4)	0.00 (1)	0.00 (1)
COSSR5	0.78 (6)	0.68 (4)	0.16 (2)	0.50 (3)	0.50 (3)
Average	0.39	0.20	0.38	0.07	0.19

^a Nei's genetic diversity. All areas = Minas Gerais (MG) areas A1 to A18 and São Paulo (SP) areas A1 to A6. The number of alleles is shown in parentheses.

^b MG areas (A5, A17, A13, and A18) analyzed together with SP areas (A5 and A6).

internal cut ends were squeezed, and the sap was blotted onto buffered charcoal yeast extract (BCYE) agar plates (Wells et al. 1981), followed by incubation at 28°C for 15 to 30 days. The identification as *X. fastidiosa* was carried out based on in vitro fastidious growth, convex format, and whiteness of colonies, which were streaked it onto Pierce disease 3 (PD3) agar plates medium and incubated at 28°C for 7 days, following PCR assays using the RST 31/33 primers for confirmation the authenticity of bacterium.

***X. fastidiosa* typing.** The single sequence repeat (SSR) and MLST methodologies were used to type 21 strains of *X. fastidiosa* isolated from olive trees from 11 different orchards at nine geographic regions. The number of strains used for typing was defined based on proportion of successful isolation for each geographic area but with each isolate representing one infected olive plant. The bacteria were triple passaged on axenic PD3 medium, and total DNA was extracted using the Wizard DNA extraction kit (Promega Corporation, Madison, WI). Twelve previously described SSR loci (Francisco et al. 2017; Lin et al. 2005) were grouped in four sets, and the amplifications were optimized to amplify *X. fastidiosa* DNA using multiplexed primers (Supplementary Table S1). The primer sets were selected based on subspecies specificity of SSR in *X. fastidiosa* (Coletta-Filho et al. 2014), as well as the presence of multiple peaks based on previous tests. Before fragment analysis, the reactions were diluted 20× in sterile milli-Q water, 1 µl was added into 10 µl of Hi-Di formamide and 0.1 µl of the GeneScan 500-LIZ Size Standard (both from Life Technologies, Foster City, CA), and the mixture was loaded onto the gel. Capillary electrophoresis was run on an ABI 3730XL DNA sequencer (Life Technologies), and the peak size in base pairs was estimated by Peak Scanner software (version 1.0; Life Technologies).

For the MLST methodology, seven loci (*leuA*, *petC*, *malF*, *cysG*, *holC*, *nuoL*, and *glT*) were amplified from triple passage of *X. fastidiosa* cells in axenic medium using the same methodology reported by Yuan et al. (2010). An aliquot of the reaction was used to confirm of the amplicon size in agarose gels (1%), and the remaining sample (approximately 20 µl) was used for PCR cleanup using the ExoSAP- IT kit (Thermo Fisher Scientific, Waltham, MA). The amplicon was directly sequenced on both strands (ABI 3130 sequencer; Life Technologies). The forward and reverse

sequences of each isolate were assembled by the CLC Genomics Workbench platform (Qiagen, Hilden, Germany). Single-nucleotide polymorphisms (SNPs) were manually checked on the electropherograms.

Genetic analysis. The allele size for each SSR locus was used to define the haplotypes within the set of *X. fastidiosa* isolates. The same multilocus microsatellite genotypes (MLMGs) were considered as a clone or haplotype as determined by GENODIVE, version 2.0b23 (Meirmans and Van Tienderen 2004). Nei's measure of genetic diversity corrected for the population size was calculated as $H = [(1 - \sum p_i^2) \times (n/n - 1)]$, where p_i was the frequency of allele i at the locus and n was the number of individuals (Nei 1978) determined using the SSR locus results. The principal coordinate analysis (PCoA) was used to plot patterns within a multivariate SSR dataset (e.g., multiple loci and multiple samples) by using GeneAlex, version 6.5 (Peakall and Smouse 2012).

Reference sequences for each MLST locus was downloaded from the *X. fastidiosa* MLST database (<https://pubmlst.org/xfastidiosa/>) and aligned with the sequences obtained in this work using Mega v.6.06 software (Tamura et al. 2013). The MLST profile of each isolate was defined using the information described in the *X. fastidiosa* MLST database (<https://pubmlst.org/xfastidiosa/>). The identification of a new allele for each MLST locus, as well as the construction of a new allelic profile, which was assigned a sequence type (ST) number, were performed according to the instructions found at <https://pubmlst.org/xfastidiosa> and generously provided by the curator of MLST database, Dr. Lenard Nunney (University of California, Riverside). Concatenated sequences of the seven MLST loci were used to determine the genetic relationships among all known *X. fastidiosa* strains and novel STs obtained in this study. The phylogenetic reconstructions were based on Bayesian interferences using the Markov chain Monte Carlo method with 100,000 interactions and 1,000 bootstraps using MrBayes v.3.2 (Huelsenbeck and Ronquist 2001). Hasegawa-Kishino-Yano with an invariable site and a γ -distributed rate (HKY +I+G) was defined as the best-fit model of DNA evolution by PAUP v.4 (Swofford 2002) and used in this analysis. To represent the genetic relationship among *X. fastidiosa* STs, we built a NeighborNet phylogenetic network using SplitTree, v.4 (Huson and

TABLE 3. Multilocus sequence typing (MLST) allelic profiles obtained for *Xylella fastidiosa* subsp. *pauca* strain isolates from olive trees with olive quick decline syndrome symptoms

Strain	Origin ^b	Olive variety	Age (years)	Data isolation	MLST profile ^a							ST ^c
					<i>leuA</i>	<i>petC</i>	<i>malF</i>	<i>cysG</i>	<i>holC</i>	<i>nuoL</i>	<i>glT</i>	
XF15.3	MG, A1	Negroa	12	January 2017	7	6	8	10	11	8	8	16
XF64.2	MG, A1	Coratina	12	January 2017	7	6	8	10	11	8	8	16
XF71.2	MG, A1	Rodapés	12	January 2017	7	6	8	10	11	8	8	16
XF188.4	MG, A2	Grappolo	9	April 2017	7	6	8	10	11	8	8	16
XF189.2	MG, A2	Grappolo	9	April 2017	7	6	8	10	11	8	8	16
XF190.1	MG, A2	Grappolo	9	April 2017	7	6	8	10	11	8	8	16
XF191.2	MG, A2	Grappolo	9	April 2017	7	6	8	10	11	8	8	16
XF217.1	MG, A4	Arbequina	9	April 2017	7	6	8	10	11	8	8	16
XF221.2	MG, A4	Arbequina	9	April 2017	7	6	8	10	11	8	8	16
XF282.1	MG, A5	Grappolo	10	January 2018	7	6	8	10	11	8	8	16
XF297.1	MG, A13	Grappolo	8	January 2018	7	6	8	10	11	8	8	16
XF308.1	MG, A18	Grappolo	9	October 2017	7	6	8	10	11	8	8	16
XF319.2	MG, A17	Unknown	9	November 2017	7	6	8	10	11	8	8	16
XF156.1	SP, A1	Grappolo	11	March 2017	7	6	7	34	10	20	8	84
XF363.1	SP, A1	Grappolo	11	December 2017	7	6	7	34	10	20	8	84
XF369.2	SP, A1	Grappolo	11	December 2017	7	6	7	34	10	20	8	84
XF367-1	SP, A1	Grappolo	11	December 2017	7	6	8	10	11	20	8	86
XF143.2	SP, A2	Arbosana	6	February 2017	7	6	8	10	11	8	8	16
XF263.2	SP, A2	Grappolo	6	May 2017	7	6	8	10	11	8	8	16
XF271.2	SP, A6	Ascolana	7	September 2017	7	6	8	10	11	8	8	16
XF168.1	SP, A5	Koroneiki	7	April 2017	7	6	8	10	10	8	8	85

^a MLST loci amplified from total DNA of *X. fastidiosa* colonies isolate from olive trees. Numbers identify the different alleles. Novel alleles (20 and 34) are shown in italics.

^b Areas (orchards) in the Minas Gerais (MG) and São Paulo (SP) States (southeastern Brazil) used for *X. fastidiosa* isolation.

^c Sequence type (STs). Novel STs (84, 85, and 86) are shown in bold. Numbers identify the alleles. For more information, visit the *Xylella fastidiosa* MLST Databases website (<https://pubmlst.org/xfastidiosa/>).

Bryant 2006). The genetic distances among *X. fastidiosa* STs were inferred using the neighbor-joining method implemented in Mega v.6.06 software (Tamura et al. 2013). The evolutionary distances were computed using HKY+I+G and 1,000 bootstraps. The genetic relationship of the new alleles found for the genes *cysG* and *nuoL* were also reconstructed using the neighbor-joining method with 1,000 bootstraps implemented in Mega v.6.06 software.

Nucleotide sequence accession numbers. All MLST data are available at the *X. fastidiosa* MLST database. The gene sequences for the novel MLST alleles obtained in the present study are available at GenBank under the following accession numbers: *cysG* allele 34 = MH706701 and *nuoL* allele 20 = MH706702.

RESULTS

Distribution of *X. fastidiosa* and symptoms. In total, 24 different olive orchards located in geographic regions with altitudes ranging from 800 to 1,780 m were evaluated for the presence of disease symptoms similar to those described for OQDS. Samples (*n* = 395) were collected from diseased branches and the asymptomatic portions were used for diagnosis. *X. fastidiosa* was detected in 20 of 24 sampled orchards (83%) against a successful isolation of 55.1% (13 of 24 orchards) (Table 1; Supplementary Fig. S1). Although the olive tree samples from Minas Gerais areas A6, A7, A8, A14, A15, and A17 were PCR positive for *X. fastidiosa*, the bacterial isolation in artificial culture medium was unsuccessful. On the other hand, PCR-negative diagnosis was obtained for some samples from Minas Gerais areas A10, A13, and A18 but the isolation of *X. fastidiosa* was very successful (Table 1). Taking into account the number of sampled trees, the overall *X. fastidiosa* incidence was 43.7%, considering both PCR and axenic isolation methods, which means that regular leaf scorch is not a specific OQDS symptom in Brazil. Moreover, 82% of the samples with a positive diagnosis for *X. fastidiosa* showed OQDS foliar symptoms identical to those previously described in southern Italy (Saponari et al. 2017) such as leaves wilting followed by scorching from the apex to the base and, later on, general leaf scorching, always restricted to a few branches on the canopy at the beginning of the disease. Another 18% of the *X. fastidiosa*-positive samples showed wilted or yellowish-colored leaves but no desiccated leaves, which could denote the initial stages of infection (Supplementary Fig. S2).

Samples from Minas Gerais areas A1 to A4 corresponded to 34% of the total analyzed as a consequence of the higher number of OQDS-like symptomatic plants, resulting in an infection rate of 2.7 to 84.2% (Table 1). Although 100% of *X. fastidiosa* infections were obtained for samples collected in Minas Gerais areas A8, A15, and A17 and São Paulo area A3, the limited number of sampled plants in these areas could have introduced bias into the diagnosis (Table 1).

At the same time, the few samples taken from Minas Gerais A11 and A16 areas and from São Paulo A5 could have introduced bias on the negative results for presence of *X. fastidiosa* in these areas.

***X. fastidiosa* diversity and phylogenetic analysis.** Of the 12 SSR loci used, only two (CSSR42 and GSSR12) were monomorphic. On the other hand, the amplification of loci COSSR4, OSS17, and COSSR5 resulted in highly polymorphic alleles for all *X. fastidiosa* strains independently of the geographic origin of the pathogen, totalizing 21, 20, and 18 different alleles, respectively (Table 2). Overall, the genetic diversity was H_{Nei} = 0.39 and the total number of alleles was *n* = 56. Strains isolated from São Paulo area A1 showed the highest values for genetic diversity (H_{Nei} = 0.38) and number of different alleles (*n* = 32), even compared with distinct geographic regions analyzed together such as Minas Gerais areas A5, A17, A13, and A18 and São Paulo areas A5 and A16, which exhibited an H_{Nei} = 0.18. A similar value was obtained with strains from Minas Gerais areas A1 to A4 (H_{Nei} = 0.20), and the lowest diversity index (H_{Nei} = 0.07) was found for strains isolated from São Paulo area A2 (Table 2). PCoA segregated the strains in only one cluster, comprising 85.7% of strains, except XF156.1, XF367.1, and XF369.2, from São Paulo A1, which were placed outside the cluster.

The MLST analysis showed that strains characterized as ST16 was frequently found in the majority of the areas analyzed, characterizing a clonal dispersion of the pathogen, except for the areas A1 and A5 in São Paulo State, where three novel STs—ST84 and ST86 (A1) and ST85 (A5)—were obtained (Table 3). For ST84 and ST86, a new allele was identified for the locus *nuoL* (allele 20) based on one SNP, which resulted in the substitution of C by T at position 113 (Supplementary Table S3). The new allele (number 34) of locus *cysG* was characterized by three SNPs observed at positions 266 (substitution of C by T), 522 (G by A), and 587 (replacing C or G by A) (Table 4). Evidence for a recombination event was observed for this locus at position 389, as confirmed by both DNAsp and GARD software. No new allele was observed for ST85. Phylogenetic analysis clustered both novel alleles for *cysG* (number 34) and *nuoL* (number 20) loci together with the alleles from *X. fastidiosa* subsp. *pauca* from citrus and coffee plants (Fig. 1A and B). Concatenated sequences of the seven housekeeping genes totaling 4,161 nucleotides placed the novel STs from Brazilian olive trees into the *X. fastidiosa* subsp. *pauca* clades. Surprisingly, the STs 85 and 86 grouped with ST16 (isolated from coffee and olive trees) and ST66 (isolate from coffee trees). In contrast, ST84 was closely related to STs from *X. fastidiosa* causing citrus disease in Brazil and Argentina and also almond disease in Argentina (ST86). A clear split can be observed between the STs originating from South and Central America clades formed by the *X. fastidiosa* subsp. *pauca* strains (Fig. 2, dashed circles), even when a common host such as coffee was shared between both continents.

TABLE 4. Single-nucleotide polymorphisms and potential recombination sites among the *Xylella fastidiosa* subsp. *pauca* strains for the *cysG* locus

Allele	Sequence types ^b	Position in <i>cysG</i> sequence (total of 606 pb) ^a																			
		023	028	030	068	071	083	135	159	186	206	209	216	236	237	238	266	276	281	282	284
9	11, 12, 13, 64, 65, 69, 78	C	G	T	C	C	C	T	G	G	A	C	G	C	A	G	C	G	G	C	G
10	16, 66, 85**, 86**	A	G	T	C	C	C	T	G	G	A	C	G	C	A	G	C	G	G	C	G
11	14, 68, 70, 71	C	A	T	C	C	A	C	A	G	A	C	G	C	A	G	C	G	G	C	G
24	53	C	A	T	C	C	A	C	A	A	G	T	C	C	G	C	C	G	G	T	G
27	73	C	A	C	C	C	A	C	A	G	G	T	G	T	G	C	C	A	G	C	A
28	74	C	A	T	T	T	C	C	A	G	G	T	G	C	G	C	C	G	A	C	G
31	80	C	A	T	C	C	A	C	A	G	G	T	G	T	G	C	C	A	G	C	A
34	84**	C	A	T	C	C	A	T	G	G	A	C	G	T	G	C	T	G	A	C	G

(Continued on next page)

^a The most homologous sequences are shown in bold. Column 389 indicates the recombination region (395 bp) according to DNAsp and GARD implemented in DataMonkey. Bold and underlined letters indicate nucleotide polymorphism that characterizes the novel allele.
^b Double asterisks (**) indicate novel sequence types.

DISCUSSION

Outbreaks in olive trees of infection by *X. fastidiosa* both in southern Italy in 2013 and southeast Brazil in 2016 (São Paulo and Minas Gerais States) were caused by different strains of *X. fastidiosa* subsp. *pauca* classified as ST53 (Elbeaino et al. 2014), and ST16 (Coletta-Filho et al. 2016), respectively, according to MLST methodology. Both STs had coffee plants as a common host but ST53 was also found to infect oleander plants (*Olea oleaster*) in Central America (Nunney et al. 2014) and ornamental plant species in southern Italy beyond olive trees (Loconsole et al. 2016). Recently, novel STs belonging to subsp. *pauca* were intercepted in Europe (ST73 and ST74) in coffee plants, most likely originating from Central America (Jacques et al. 2016; Loconsole et al. 2016). Another subsp. *pauca* ST was found in oleander plants (*Nerium oleander*) in the Balearic Islands (ST80) (European Food Safety Authority 2018). Together, these results definitively change the phylogeographical and host restrictions of *X. fastidiosa* subsp. *pauca* to citrus and coffee plants in South America and highlight the expansion of this subspecies to new hosts and environments. These novel hosts also give rise to a discussion regarding the *pauca* nomenclature proposed by Schaad et al. (2004) based on the narrow host range of this bacterium (*pau'ca* L. fem. adj. *pauca* few).

The movement of asymptomatic but infected plant material from areas wherein the pathogen occurs to environments that are ecologically prone to harboring and spreading the bacteria to potential hosts was the main factor underlying the movement of *X. fastidiosa* subsp. *pauca* to a new geographic scenario (i.e., Europe) (Almeida and Nunney 2015). Furthermore, the introduction of an exotic vector-borne phytopathogen may also favor, for the first years, a clonal wave, spreading dispersion of the pathogen, as observed for the outbreak of *X. fastidiosa* (ST53) in southern Italy (Bleve et al. 2016; Elbeaino et al. 2014; Mang et al. 2016) or 'Candidatus Liberibacter asiaticus' psyllid-vectored bacteria in Brazil (H. D. Coletta-Filho, personal communication). However, because *X. fastidiosa* is naturally competent (Kung and Almeida 2014), the presence of a local pathogen reservoir plays an important role for intra- or intersubspecific homologous recombination (Barbosa et al. 2015; Nunney et al. 2012), which may contribute to genome evolution and adaptation to new hosts (Coletta-Filho et al. 2017; Nunney et al. 2014).

Commercial olive farming in southeast Brazil began approximately in the 2000s in areas with high altitudes (800 to 1,900 m) and mild temperatures, favoring flowering and fructification. This recent agricultural activity is spread over the Serra da Mantiqueira and neighboring areas for approximately 2,000 ha. Most of these areas are surrounded by native vegetation (Table 1), with minimal spray of insecticides for pest management, scenarios that could potentialize the vector's diversity and population size (Froza 2017). Despite a recent report of *X. fastidiosa* in olive plants in southeastern Brazil, 83% of the sampled orchards were infested by the bacteria as observed by PCR or isolation, although the frequency of positive samples was variable in

individual orchards (Table 1). One hypothesis for the long-distance dispersion of *X. fastidiosa* could be anthropogenic action using infected vegetative material. Supporting this first statement, we determined that the vertical transmission of *X. fastidiosa* by rooting asymptomatic branches sampled from symptomatic olive plants was approximately 13% (data not shown). Additionally, a unique ST (ST16) was assessed in 15 of 20 typed strains and was present in seven of nine geographic regions (Table 3). However, a natural reservoir for the ST16 of *X. fastidiosa* located near the sampled orchards cannot be discarded and needs to be explored in future works.

Beyond ST16, we found three new STs related to *X. fastidiosa* subsp. *pauca* colonizing OQDS-symptomatic olive trees in two areas of São Paulo State (A1 and A5). Interestingly, area A1 hosted two different STs (ST84 and ST86) identified in the four *X. fastidiosa* strains analyzed. ST84 represents a novel ST with two novel alleles for the MLST loci *cysG* number 34 and *nuoL* number 20, which have not been described yet. However, two other alleles in the ST84 (*malF* number 7 and *holC* number 10) are exclusive to *X. fastidiosa* subsp. *pauca* associated with citrus variegated chlorosis in Brazil, and the three others alleles are from coffee-associated *X. fastidiosa* (<https://pubmlst.org/xfastidiosa/>; Coletta-Filho et al. 2017). The ST86 profile comprises all MLST loci from ST16 except for the novel allele *nuoL* number 20, while ST85 differs from ST16 only by the presence of the allele *holC* number 10 (Table 3). The allele combinations observed for ST84 (Table 4) support the intraspecies recombination hypothesis initially proposed by Almeida et al. (2008), which was later confirmed (Coletta-Filho et al. 2017; Nunney et al. 2012), where the ST (strains) from coffee plants are the potential donor subspecies.

The host jump of *X. fastidiosa* from coffee to olive plants, probably carried by xylem-sap-feeder sharpshooters following anthropogenic activity which may have unwittingly spread the bacteria through contaminated vegetative material, is a plausible hypothesis to explain the prevalence of ST16 in the symptomatic olive trees. Supporting this hypothesis, PCoA using the SSR loci placed the majority *X. fastidiosa* strains into a unique cluster, independent of geographic origin, except the strains characterized as ST84 from São Paulo A1 (Fig. 3). A local natural reservoir located near the olive orchards could also contribute to genotypic diversification on a specific area such as found in São Paulo areas A1 and A5. No known *X. fastidiosa* subsp. *pauca* reservoir such as infected coffee and citrus orange plants was found close to olive orchards in areas A1 and A5. Several species of native host plants were found to host *X. fastidiosa* infection but with miscellaneous or no visible symptoms (Purcell and Saunders 1999). On the other hand, asymptomatic plants are more attractive for the vectors and provide an effective inoculum sources for *X. fastidiosa* acquisition and spread (Daugherty et al. 2017; Marucci et al. 2005). In addition, the variability of vector populations also must be considered a potential driver for genetic variability. Coincidentally, the Shannon index for genetic diversity of leafhoppers species collected by yellow

TABLE 4. (Continued from previous page)

Allele	Position in <i>cysG</i> sequence (total of 606 pb) ^a																	
	303	323	335	348	349	371	380	389	476	503	510	514	521	522	548	554	587	599
9	G	A	T	A	A	C	T	G	G	A	G	G	G	C	G	C	C	G
10	G	A	T	A	A	C	T	G	G	A	G	G	G	C	G	C	C	G
11	G	A	T	A	A	C	T	G	C	G	G	G	G	T	G	T	G	G
24	A	G	C	A	G	C	C	G	C	G	G	G	A	T	G	C	G	A
27	A	G	C	A	G	A	C	T	G	A	G	G	G	C	G	C	C	G
28	A	G	C	G	G	C	C	T	C	G	A	A	G	C	G	C	G	G
31	A	G	C	A	G	A	C	T	G	A	G	G	G	C	G	C	C	G
34	A	G	C	A	A	C	C	G	C	G	G	G	G	C	A	C	A	G

Fig. 1. Single-locus phylogenetic trees placing the new alleles **A**, *cysG* and **B**, *nuoL* found in *Xylella fastidiosa* subsp. *pauca* strains infecting olive trees in Brazil. Circles show the different plants species where the *X. fastidiosa* strains with *cysG* and *nuoL* alleles were isolated. Numbers indicate bootstrap support; trees generated by Mega v.6.06 software (Tamura et al. 2013).

Fig. 2. Neighbor-net reconstruction of relationships among sequence types (STs) of *Xylella fastidiosa* infecting olive trees in southeast Brazil in relation to all currently known STs. Circles show the different plants species affected by *X. fastidiosa* subsp. *pauca* in South America. Within the subsp. *pauca* cluster, the dashed circles group STs according to their geographic origin (South or Central America), independently of hosts.

Fig. 3. Principal coordinate analysis of single sequence repeat markers obtained for *Xylella fastidiosa* strains isolated from olive trees in southeast Brazil. Squares represent strains from São Paulo A1, triangles from São Paulo A2, diamonds from Minas Gerais A1 to A4, and “x” from other regions.

trap was significantly higher for São Paulo area A1 compared with other areas included in this article (Froza 2017).

The geographic origin of *X. fastidiosa* subsp. *pauca* is still uncertain but South America is suggested as a possible center of origin, with later introduction into Central America (Nunney et al. 2014), supported by placement of strains into two major clades (Fig. 2), consistent with results previously obtained by Giampetruzzi et al. (2017). Recently, the number of known STs related to *X. fastidiosa* subsp. *pauca* in South America strongly increased from five (STs 11, 12, 13, 14, and 16) (Nunney et al. 2012) to nine (STs 64, 65, 66, 68, 69, 70, and 71) Coletta-Filho et al. 2017) and, now, three new STs (STs 84, 85, and 86), which reinforce this hypothesis.

Analysis of the dataset from more variable genomic regions (SSRs) indicates 20 different MLMGs of 21 analyzed strains (Supplementary Table S2), whereas only four STs were observed. Such differences were also observed by Almeida et al. (2008) and Yuan et al. (2010). Overall, the genetic diversity index observed for *X. fastidiosa* subsp. *pauca* from olive ($H_{Nei} = 0.39$) was lower than those of subsp. *pauca* strains from citrus ($H_{Nei} = 0.46$) (Coletta-Filho et al. 2014) or coffee ($H_{Nei} = 0.60$) (Francisco et al. 2017). This result can be interpreted as the consequence of a recent outbreak of OQDS (Table 2). The higher diversity index obtained for the strains from São Paulo A1 ($H_{Nei} = 0.38$) agreed with richness of the MLST alleles resulting in two novel STs (84 and 86) for samples from this region.

In the past 5 years, the phylogeographic map and host range of *X. fastidiosa* subsp. *pauca* has changed considerably. In this article, we confirmed that, thus far, only *X. fastidiosa* subsp. *pauca* was found in olive plants with OQDS symptoms in southeastern Brazil, despite the recent report that *X. fastidiosa* subsp. *pauca*-infected plants were widespread over the sampled geographic regions. In addition, strains with a clonal MLST profile (ST16) and similar SSR pattern were widely distributed through the olive orchards, with few exceptions (Table 3; Fig. 3). These findings highlight the importance of exploring the population diversity in *X. fastidiosa* subsp. *pauca* to better understand the spatial distribution in olive orchards and also the probable mode of dispersion. Because of the high incidence and widespread distribution of *X. fastidiosa* subsp. *pauca* in olive in southeastern Brazil, it is necessary to implement a strategy of management of OQDS similar to that implemented for citrus variegated chlorosis in Brazil (Almeida et al. 2014).

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LITERATURE CITED

Almeida, R. P. P., Coletta-Filho, H. D., and Lopes, J. R. S. 2014. *Xylella fastidiosa*. Pages 841-850 in: Manual of Security Sensitive Microbes and Toxins. D. Liu, ed. CRC Press, Taylor & Francis Group, LLC, Boca Raton, FL.

- Almeida, R. P. P., Nascimento, F. E., Chau, J., Prado, S. S., Tsai, C. W., and Lopes, S. A. 2008. Genetic structure and biology of *Xylella fastidiosa* strains causing disease in citrus and coffee in Brazil. Appl. Environ. Microbiol. 74:3690-3701.
- Almeida, R. P. P., and Nunney, L. 2015. How do plant diseases caused by *Xylella fastidiosa* emerge? Plant Dis. 99:1457-1467.
- Barbosa, D., Alencar, V. C., Santos, D. S., Oliveira, A. C. de F., de Souza, A. A., and Coletta-Filho, H. D. 2015. Comparative genomic analysis of coffee-infecting *Xylella fastidiosa* strains isolated from Brazil. Microbiology 161:1018-1033.
- Bleve, G., Marchi, G., Ranaldi F., Gallo, A., Cimaglia F., Logrieco A.F., Mita, G., Ristori J., and Surico G. 2016. Molecular characteristics of a strain (Salento-1) of *Xylella fastidiosa* isolated in Apulia (Italy) from an olive plant with the quick decline syndrome. Phytopathol. Mediterr. 55:139-146.
- Coletta-Filho, H. D., Francisco, C. S., and Almeida, R. P. P. 2014. Temporal and spatial scaling of the genetic structure of a vector-borne plant pathogen. Phytopathology 104:120-125.
- Coletta-Filho, H. D., Francisco, C. S., Lopes, J. R. S., de Oliveira, A. F., and da Silva, L. F. O. 2016. First report of olive leaf scorch in Brazil associated with *Xylella fastidiosa* subsp. *pauca*. Phytopathol. Mediterr. 55:130-135.
- Coletta-Filho, H. D., Francisco, C. S., Lopes, J. R. S., Muller, C., and Almeida, R. P. P. 2017. Homologous recombination and *Xylella fastidiosa* host-pathogen associations in South America. Phytopathology 107:305-312.
- Daugherty, M. P., Zeilinger, A. R., and Almeida, R. P. P. 2017. Conflicting effects of climate and vector behavior on the spread of a plant pathogen. Phytobiomes 1:46-53.
- Elbeaino, T., Valentini, F., Aboukubaa, R., Moubarak, P., Yaseen, T., and Digiario, M. 2014. Multilocus sequence typing of *Xylella fastidiosa* isolated from olive affected by Olive Quick Decline Syndrome in Italy. Phytopathol. Mediterr. 53:533-542.
- European Food Safety Authority. 2016. Update of a database of host plants of *Xylella fastidiosa*: 20 November 2015. EFSA J. 14:4378.
- European Food Safety Authority. 2018. Updated pest categorisation of *Xylella fastidiosa*. EFSA J. 16:5357.
- Francisco, C. S., Ceresini, P. C., Almeida, R. P. P., and Coletta-Filho, H. D. 2017. Spatial Genetic Structure of Coffee-Associated *Xylella fastidiosa* populations indicates that cross infection does not occur with sympatric citrus orchards. Phytopathology 107:395-402.
- Froza, J. A. 2017. Levantamento de espécies de cigarrinhas (Hemiptera: Auchenorrhyncha) com ênfase em possíveis vetores de *Xylella fastidiosa* em pomares de oliveira na Serra da Mantiqueira. Online publication. <http://www.teses.usp.br/teses/disponiveis/11/11146/tde-14032018-182216/pt-br.php>
- Giampetruzzi, A., Saponari, M., Loconsole, G., Boscia, D., Savino, V. N., Almeida, R. P. P., Zica, S., Landa, B. B., Chacón-Díaz, C., and Saldarelli, P. 2017. Genome-wide analysis provides evidence on the genetic relatedness of the emergent *Xylella fastidiosa* genotype in Italy to isolates from Central America. Phytopathology 107:816-827.
- Huelsenbeck, J. P., and Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754-755.
- Hufbauer, R. A., Facon, B., Ravigné, V., Turgeon, J., Foucaud, J., and Lee, C. E. 2012. Anthropogenically induced adaptation to invade (AIAD): Contemporary adaptation to human-altered habitats within the native range can promote invasions. Evol. Appl. 5:89-101.
- Huson, D. H., and Bryant, D. 2006. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. 23:254-267.
- Jacques, M. A., Denancé, N., Legendre, B., Morel, E., Briand, M., and Mississippi, S. 2016. New coffee plant-infecting *Xylella fastidiosa* variants derived via homologous recombination. Appl. Environ. Microbiol. 82:1556-1568.

- Kung, S. H., and Almeida, R. P. P. 2014. Biological and genetic factors regulating natural competence in a bacterial plant pathogen. *Microbiology* 160: 37-46.
- Lin, H., Civerolo, E. L., Hu, R., Barros, S., Francis, M., and Walker, M. A. 2005. Multilocus simple sequence repeat markers for differentiating strains and evaluating genetic diversity of *Xylella fastidiosa*. *Appl. Environ. Microbiol.* 71:4888-4892.
- Loconsole, G., Saponari, M., Boscia, D., D'Attoma, G., Morelli, M., and Martelli, G. P. 2016. Intercepted isolates of *Xylella fastidiosa* in Europe reveal novel genetic diversity. *Eur. J. Plant Pathol.* 146:85-94.
- Mang, S. M., Frisullo, S., Elshafie, H. S., and Camele, I. 2016. Diversity evaluation of *Xylella fastidiosa* from infected olive trees in Apulia (Southern Italy). *Plant Pathol. J.* 32:102-111.
- Marucci, R. C., Lopes, J. R. S., Vendramim, J. D., and Corrente, J. E. 2005. Influence of *Xylella fastidiosa* infection of citrus on host selection by leafhopper vectors. *Entomol. Exp. Appl.* 117:95-103.
- Meirmans, P. G., and Van Tienderen, P. H. 2004. GENOTYPE and GENODIVE: Two programs for the analysis of genetic diversity of asexual organisms. *Mol. Ecol. Notes* 4:792-794.
- Minsavage, G. V., Thompson, C. M., Hopkins, D. L., Leite, R., and Stall, R. E. 1994. Development of a polymerase chain reaction protocol for detection of *Xylella fastidiosa* in plant tissue. *Phytopathology* 84:456-461.
- Murray, M. G., and Thompson, W. F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* 8:4321-4326.
- Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89:583-590.
- Nunney, L., Ortiz, B., Russell, S. A., Sánchez, R. R., and Stouthamer, R. 2014. The complex biogeography of the plant pathogen *Xylella fastidiosa*: Genetic evidence of introductions and subspecific introgression in central America. *PLoS One* 9:e112463.
- Nunney, L., Vickerman, D. B., Bromley, R. E., Russell, S. A., Hartman, J. R., and Morano, L. D. 2013. Recent evolutionary radiation and host plant specialization in the *Xylella fastidiosa* subspecies native to the United States. *Appl. Environ. Microbiol.* 79:2189-2200.
- Nunney, L., Yuan, X., Bromley, R., Hartung, J., Montero-Astúa, M., Moreira, L. 2010. Population genomic analysis of a bacterial plant pathogen: Novel insight into the origin of pierce's disease of grapevine in the U.S. *PLoS One* 5:e15488.
- Nunney, L., Yuan, X., Bromley, R. E., and Stouthamer, R. 2012. Detecting genetic introgression: High levels of intersubspecific recombination found in *Xylella fastidiosa* in Brazil. *Appl. Environ. Microbiol.* 78:4702-4714.
- Peakall, R., and Smouse, P. E. 2012. GenALEX 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—An update. *Bioinformatics* 28:2537-2539.
- Purcell, A. 2013. Paradigms: Examples from the bacterium *Xylella fastidiosa*. *Annu. Rev. Phytopathol.* 51:339-356.
- Purcell, A. H., and Saunders, S. R. 1999. Fate of Pierce's disease strains of *Xylella fastidiosa* in common riparian plants in California. *Plant Dis.* 83: 825-830.
- Saponari, M., Boscia, D., Altamura, G., Loconsole, G., Zicca, S., and D'Attoma, G. 2017. Isolation and pathogenicity of *Xylella fastidiosa* associated to the olive quick decline syndrome in southern Italy. *Sci. Rep.* 7: Article 17723.
- Schaad, N. W., Postnikova, E., Lacy, G., Fatmi, M., and Chang, C.-J. 2004. *Xylella fastidiosa* subspecies: *X. fastidiosa* subsp. *piercei* subsp. nov., *X. fastidiosa* subsp. *multiplex* subsp. nov., and *X. fastidiosa* subsp. *pauca* subsp. nov. *Syst. Appl. Microbiol.* 27:290-300.
- Schuenzel, E. L., Scally, M., Stouthamer, R., and Nunney, L. 2005. A multi-gene phylogenetic study of clonal diversity and divergence in North American strains of the plant pathogen *Xylella fastidiosa*. *Appl. Environ. Microbiol.* 71:3832-3839.
- Sicard, A., Zeilinger, A. R., Vanhove, M., Schartel, T. E., Beal, D. J., Daugherty, M. P. 2018. *Xylella fastidiosa*: Insights into an emerging plant pathogen. *Annu. Rev. Phytopathol.* 56:1-22.
- Swofford, D. L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), version 4. Sinauer Associates, Sunderland, MA.
- Tamura, K., Stecher, G., Peterson, D., Filipowski, A., and Kumar, S. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30:2725-2729.
- Wells, J. M., Raju, B. C., Nyland, G., and Lowe, S. K. 1981. Medium for isolation and growth of bacteria associated with plum leaf scald and phony peach diseases. *Appl. Environ. Microbiol.* 42:357-363.
- Yuan, X., Morano, L., Bromley, R., Spring-Pearson, S., Stouthamer, R., and Nunney, L. 2010. Multilocus sequence typing of *Xylella fastidiosa* causing Pierce's disease and oleander leaf scorch in the United States. *Phytopathology* 100:601-611.