INTRODUCTION

Cicadomorpha is an infraorder of Hemiptera, including approximately 35,000 described species. The species are grouped into three superfamilies based on morphological criteria: Cicadoidea (cicadas), Cercopoidea (spittlebugs and froghoppers) and Membracoidea (leafhoppers, sharpshooters and treehoppers) (Dietrich, 2005). Several species of this infraorder may injure plants directly through feeding (Atakan, 2009; Backus, 1988; Backus, Serrano, & Ranger, 2005). The direct damage, resulting in loss of sap, can be disastrous in periods of drought (Ossiannilsson, 1978). However, the indirect damage of Cicadomorpha in plants through transmission of
pathogens (Maramorosch & Harris, 1979; Nielson, 1968) is by far the most important economic impact of those insect species.

The most serious case of pathogen transmission in Europe from Cacodromorpha in recent years is the bacterium Xylella fastidiosa. Wells (Xanthomonadales: Xanthomonadaceae). The bacterium was firstly reported in Europe in Apulia of south Italy causing the olive quick decline syndrome (OQDS) in olive trees showing severe symptoms of leaf scorch and dieback (Saponari et al., 2014). This first detection was followed by findings of several subspecies and strains of X. fastidiosa in France, Germany, Spain, and Portugal (Crusaud et al., 2018; Denancé et al., 2017, EPPO, 2016, 2019; Ols et al., 2017). X. fastidiosa is exclusively transmitted by Cacodromorpha xylem sap-feeding insects. They have sucking mouthparts (mandibular and maxillary styles) that allow them to reach the xylem of plants, from which they ingest sap (EFSA, 2013). The insect vectors of the bacterium are mainly sharpshooters (Cicadellidae, subfamily Cicadellinae) and spittlebugs (Aphrophoridae and Cercopidae; Redak et al., 2004).

Sharpshooters are the most important vectors of X. fastidiosa in the Americas, but only a few species are present in Europe. In contrast, a relatively high number of spittlebug species, which are less important vectors in America, occur in Europe (EFSA, 2015).

The chemical control of the bacterium is not possible, and the only way to slow the fast spread of disease is the use of resistant varieties, cultural and hygienic measures and chemical and biological vector control (Janse & Obradovic, 2010). Therefore, the knowledge of the vectors’ species as well as the robust understanding of their biology and ecology is necessary for the development of efficient control management practices. However, the faunal and ecological investigations of Cacodromorpha in Greece are limited. Drosopoulos, Asche, and Hoch (1986) in a thorough review of Greek Cacodromorpha recorded approximately 420 species belonging to four families, while Thanou et al. (2018) reported another 17 species of the family Cicadellidae. In respect of the potential vectors of X. fastidiosa, the former authors stated that in Greece 19 species of spittlebugs (13 and six species of the families Aphrophoridae and Cercopidae, respectively) and three species of sharpshooters are present. However, those surveys were conducted in various habitats. Given the fact that X. fastidiosa pose a serious threat especially for olive cultivation in Greece, the present work aims to investigate which species of this infraorder are present in Greek olive orchards. Special interest is given to the species which are considered as vectors of the bacterium. A representative number of those insects were tested by molecular methods to examine the possible presence of X. fastidiosa.

2 MATERIALS AND METHODS

2.1 Sampling areas

In order to determine the presence of insects belonging to the infraorder Cacodromorpha, samplings were conducted in 28 olive orchards in Greece during 2 successive years (2017 and 2018). The olive orchards were distributed in 15 regional units of central and south (Mesenia, Laconia and Chania) Greece (Figure 1). In certain sampling sites, only one sampling was performed, while in others, multiple samplings were conducted. In total, 134 samplings were carried out in those orchards during the survey period. The olive groves sampled were not subjected to chemical treatments for the control of pests and diseases. Moreover, most of the orchards maintained their natural ground vegetation through most part of the year while in some others occasional grazing by sheep took place. Although there was a diversity on the wild vegetation of the olive groves sampled, the most common plant species belonged to the families Poaceae (Avena sterilis, Hordeum spp.), Asteraceae (Crepis spp., Sonchus oleraceus) and Fabaceae (Medicago spp., Trifolium spp.).

2.2 Sampling of insects

In each site, samplings for adults were conducted with an entomological sweep net (38 cm diameter). For each orchard, five samples were taken from the canopy of olive trees and another five from the natural vegetation beneath the canopy. Each sample from the canopy consisted of 12 sweeps performed on six olive plants. Each olive tree’s canopy was swept twice with the net. A total of 30 trees were sampled per sampling site and date. Each sample on wild plants of the natural vegetation was conducted by 10 consecutive sweeps on the ground vegetation. Each sweep was performed by moving the capturing net in an arrow of 180 degrees. The overall content of the sweeping net was emptied in a plastic bag, properly labelled and sealed. All samples were frozen at –20°C, and then, targeted insects were separated in the laboratory and were conserved in ethanol 98% (EtOH) until identification. Samples were examined under a microscope for species identification which was based on appropriate keys and illustrations (Biedermann & Niedrinhous, 2009; Dmitriev, 2018; Holzinger, Kamerlander, & Nickel, 2003; Ossiannilsson, 1981, 1983; Wilson, Stewart, Biedermann, Nickel, & Niedrinhous, 2015).

2.3 Molecular methods

One hundred and thirty (130) individuals belonging to six species of spittlebugs, collected in 11 regional units of Greece, were tested for the hypothesis of carrying the bacterium X. fastidiosa. Specifically, these were: 59 individuals of Philaenus spumarius from Achaea, Aetolia-Acarnania, Chania, Chalkidiki, Corinthia, Attica, Kavala and Laconia; 36 individuals of Neophilaenus campestris from Achaea, Chalkidiki, Corinthia and Attica; 18 individuals of Lepronia coleoptrata from Arta, Cephallonia and Phthiotis; seven individuals of Cercopis sanguinolenta from Aetolia-Acarnania and Corinthia; five individuals of Aphrophora alni from Cephallonia; and five individuals of Philaenus signatus from Aetolia-Acarnania and Corinthia.

The protocol applied during the molecular testing is proposed by the European and Mediterranean Plant Protection Organization (EPPO, 2018). Insect heads of single individuals were used for genomic
FIGURE 1  Geographical distribution of sampling sites (olive orchards) across Greece (the colour of the circle indicates the periods that samplings were conducted) [Colour figure can be viewed at wileyonlinelibrary.com]
DNA (gDNA) extraction according to the CTAB-based extraction protocol for vectors. Two sets of primers designed previously were used for the amplification by polymerase chain reactions (PCRs). The primer pair RST31 (5'–GGTTAAATTCCGAATGGTACGTTG–3') and RST33 (5'–CACCATGCTATCCCGGTG–3') was used for the amplification of a 733-bp fragment of the rpoD gene, which codes for an RNA polymerase sigma-70 factor (EPPO, 2018; Minsavage, Thompson, Hopkins, Leite, & Stall, 1994) and the primer pair HL5 (5'–AAGGCCAATAAACGCGCACTA–3') and HL6 (5'–GGTTTTGCTGACTGGCAACA–3') for the amplification of a 221-bp fragment of the hypothetical HL protein gene (EPPO, 2018; Francis, Lin, Cabrera-La Rosa, Daddapaneni, & Civerolo, 2006).

Two microlitres of the gDNA extract was used as template in 25 µl total reactions for the amplification using the primer pair RST31/RST33. PCRs contained 0.2 mM dNTPs, 0.3 µM of each primer, 1.5 mM MgCl₂, 0.03 U/µl of Platinum Taq DNA Polymerase (Thermo Fisher Scientific) and 1x enzyme buffer. The PCR conditions applied were the following: an initial denaturation step at 94°C for 5 min; 40 cycles at 94°C for 30 s, 55°C for 30 s and 72°C for 45 s; and a step of final extension at 72°C for 7 min.

Two microlitres of the gDNA extract was used as template in a final volume of 25 µl for the amplification using the primer pair HL5/HL6. All PCRs contained 0.2 mM dNTPs, 0.3 µM of each primer, 1 unit of Kapa Taq DNA Polymerase (Kapa Biosystems) and 1x enzyme buffer. Reactions were performed under the following conditions: one step of initial denaturation at 95°C for 3 min; 35 cycles at 95°C for 30 s, 60°C for 30 s and 72°C for 1 min; and a step of final extension at 72°C for 1 min.

Suitable negative and positive controls for the bacterium X. fastidiosa were included in each PCR for both sets of primers as proposed in the EPPO diagnostic protocol (EPPO, 2018) to avoid false-negative and false-positive results. DNA from the bacterium Xanthomonas campestris was used as negative isolation control (NIC), molecular grade water was used as negative amplification control (NAC), gDNA extract from insects spiked with X. fastidiosa CoDIRO strain was used as positive isolation control (PIC), and DNA of X. fastidiosa isolated from a suspension with approximately 10⁵ cfu/ml was used as positive amplification control (PAC). All experiments were conducted with the Veriti 96-Well PCR equipment (Applied Biosystems). The amplified products were loaded and visualized on a 1.2% agarose gel containing the Midori Dye, Green.

### 3 RESULTS

During the surveys in Greek olive orchards, 4,350 insects of the infraorder Cicadomorpha were collected. From those insects, 1,026 individuals were spittlebugs (Table S1). The identification of those insects revealed the presence of five species of the family Aphrophoridae and one species of the family Cercopidae. The most frequently observed spittlebugs were P. spumarius, which was identified in 13 olive orchards distributed in nine regional units, and N. campestris, which was observed in five olive orchards distributed in five regional units. P. signatus was recorded in four orchards distributed in three regional units, while L. coleoptrata was identified in four sampling areas. A. alni and C. sanguinolenta were both recorded in two orchards (Table 1).

Cicadellidae was the most affluent family of the infraorder Cicadomorpha regarding the number of species and individuals in olive orchards. During the surveys, 3,315 insects from this family were collected and 42 species were identified (Table S2), pertaining to five subfamilies. Deltacephalinae was the most abundant subfamily being represented by 26 species, followed by Typhlocybinae, by eight species. Agallinae, Aphrodinae and Hecalinae were represented by four, three and one species, respectively. Lastly, nine adults of the family Cicadidae were collected and two species of the family were identified (Table 2). However, sweep netting is not the appropriate method for sampling cicadas, and for this reason, the results regarding this family are considered only indicative.

The molecular analysis with PCR assays using both sets of primers for the detection of X. fastidiosa revealed the absence of the bacterium in all 130 individuals tested. In addition, no PCR products were produced with negative control templates (NIC, NAC) while DNA fragments of the expected size were produced with positive control templates (PIC, PAC), for both sets of primers (Figure 2).

### 4 DISCUSSION

According to the results of our surveys, P. spumarius was the most common species of spittlebugs. A wide distribution of this species was recorded as it was found in sampling areas in central, west, north and south Greece. In most of the olive orchards, the individuals of this species were captured either in spring or in autumn. During summer months (June–September), there was an absence of this species both on ground vegetation and on the foliage of olive trees. Only in two olive groves, in Cephalonia and Laconia, adults of this species were observed during summer. However, even in those two cases only five and one individual, respectively, were captured. It has been reported that adults of P. spumarius in Spain, Portugal and Corsica tend to migrate from the olive orchards in summer and return in autumn, after the first rains during the regrowth of the ground vegetation (Cruaud et al., 2018; Morente et al., 2018). Our results strongly indicate that this phenomenon is also happening in the Greek olive orchards. Thus, it is highly possible that the distribution of P. spumarius in Greece is wider than that recorded in the present work, as in most of the olive orchards where the species was not observed, samplings were conducted only during summer months. Although most of the individuals of P. spumarius were captured on the ground vegetation, in some samplings during spring and autumn, a number of P. spumarius adults were also captured on the foliage of olive trees. N. campestris displayed a similar pattern. This species was recorded in spring and autumn, while in summer months it was absent from olive orchards. That indicates that this species also migrates during summer. It was captured mainly on ground vegetation with the exception of May and November where some individuals...
**TABLE 1**  Presence and period of capture of the species vectors of *Xylella fastidiosa* in olive orchards of Greece

| Species               | Period of capture | Attica | Euboea | Phthiotis | Aetolia-Acarnania | Achaea | Arta | Corinthia | Laconia | Messenia | Chalkidiki | Kavala | Corfu | Lefkada | Cephalonia | Chania |
|-----------------------|-------------------|--------|--------|-----------|-------------------|--------|-----|-----------|---------|----------|-----------|--------|------|--------|------------|--------|
| *Philaenus spumarius* | Spring            | x^f    | a      | –         | x^g               | –      | x^f | –         | x^g     | –        | –         | –      | –    | –      | –          | –      |
|                       | Summer            | a      | –      | a         | a                 | a      | a   | a         | a       | a        | –         | a      | a    | a      | a          | a      |
|                       | Autumn            | x^f    | –      | –         | x^f               | x^g   | –   | x^f       | –       | –        | –         | –      | –    | –      | x^g        | a      |
| *Neophilaenus campestris* | Spring        | x^f    | a      | –         | a                 | x^f   | –   | x^f       | –       | –        | –         | –      | –    | –      | x^g        | a      |
|                       | Summer            | a      | –      | a         | a                 | a     | a   | a         | a       | a        | –         | a      | a    | a      | a          | a      |
|                       | Autumn            | x^f    | –      | –         | x^f               | x^g   | –   | x^f       | –       | –        | –         | –      | –    | –      | a          | a      |
| *Philaenus signatus*  | Spring            | a      | a      | –         | x^g               | a     | –   | x^f       | –       | a        | –         | –      | –    | –      | –          | a      |
|                       | Summer            | a      | –      | a         | a                 | a     | a   | a         | a       | a        | –         | a      | a    | a      | x^f        | a      |
|                       | Autumn            | a      | –      | –         | x^f               | a     | –   | a         | a       | a        | –         | a      | a    | –      | a          | –      |
| *Lepyroia coleoptrata* | Spring            | a      | a      | –         | a                 | a     | –   | a         | –       | a        | –         | –      | –    | –      | –          | a      |
|                       | Summer            | a      | –      | x^g       | x^g               | a     | x^g | a         | a       | a        | –         | a      | a    | x^g    | a          | a      |
|                       | Autumn            | a      | –      | a         | a                 | a     | a   | a         | a       | a        | –         | a      | a    | –      | x^f        | a      |
| *Aphrophora alni*     | Spring            | a      | a      | –         | a                 | a     | -   | a         | –       | a        | –         | –      | –    | –      | –          | a      |
|                       | Summer            | a      | –      | a         | a                 | a     | a   | a         | a       | a        | –         | a      | a    | a      | x^f        | a      |
|                       | Autumn            | a      | –      | –         | x^f               | a     | –   | a         | a       | a        | –         | a      | a    | –      | a          | a      |
| *Cercopis sanguinolenta* | Spring       | a      | a      | –         | x^g               | a     | –   | x^g       | –       | a        | –         | –      | –    | –      | –          | a      |
|                       | Summer            | a      | –      | a         | a                 | a     | a   | a         | a       | a        | –         | a      | a    | a      | a          | a      |
|                       | Autumn            | a      | –      | –         | a                 | a     | –   | a         | a       | a        | –         | a      | a    | –      | –          | a      |

**Note:** –, samplings not conducted; x, presence of insect; a, absence of insect; f, capture on olive foliage; g, capture on ground vegetation; gf, foliage.

^aMonths of the capture periods: Spring: March-May; Summer: June-August; Autumn: September-December.
| Species | Attica | Euboea | Phthiotis | Aetolia-Acarnania | Achaea | Arta | Corinthia | Laconia | Messenia | Chalkidiki | Kavala | Corfu | Lefkada | Cephalonia | Chania |
|---------|--------|--------|-----------|-------------------|--------|-----|----------|--------|---------|-----------|-------|------|--------|------------|--------|
| Family: Cicadellidae | | | | | | | | | | | | | | | |
| Subfamily: Agallinae | | | | | | | | | | | | | | | |
| Anaceratagallia laevis | x | | | | | | | | | | | | | | |
| Anaceratagallia ribauti | | | | | | | | x | | | | | | | |
| Austroagallia sinuata | | | | | | | | | | x | | | | | |
| Dryodurgades reticulatus | | | | | | | | | | | | | | | x |
| Subfamily: Aphrodinae | | | | | | | | | | | | | | | |
| Anoscopus albifrons | x | | | | | | | | | | | | | | |
| Aphrodes carinatus | | | | | | | | x | | | | | | | |
| Aphrodes makarovi | | | | | | | | | | | | | | | x |
| Subfamily: Deltocephalinae | | | | | | | | | | | | | | | |
| Allygus modestus | x | | | | | | | | | | | | | | |
| Anaconura acuticeps | | | | | | | | | | | | | | | x |
| Anoplotettix fuscvenosus | | | | | | | | | | | | | | | x |
| Balclutha punctata | | | | | | | | | | | | | | | x |
| Balclutha saltuella | | | | | | | | | | | | | | | x |
| Balclutha rhenana | | | | | | | | | | | | | | | x |
| Cicadulina bipunctata | | | | | | | | | | | | | | | x |
| Doratura exilis | | | | | | | | | | | | | | | x |
| Euscelis distinguendus | | | | | | | | | | | | | | | x |
| Euscelis lineolatus | | | | | | | | | | | | | | | x |
| Euscelis ohausi | | | | | | | | | | | | | | | x |
| Exitianus capicola | | | | | | | | | | | | | | | x |
| Fieberiella sp. | | | | | | | | | | | | | | | x |
| Goniagnathus sp. | | | | | | | | | | | | | | | x |
| Macrosteles quadripunctulatus | | | | | | | | | | | | | | | x |
| Mocydia crocea | | | | | | | | | | | | | | | x |
| Neoalturus fenestratus | | | | | | | | | | | | | | | x |

(Continues)
### TABLE 2 (Continued)

| Species                        | Attica | Euboea | Phthiotis | Aetolia-Acarnania | Achaea | Arta | Corinthia | Laconia | Messenia | Chalkidiki | Kavala | Corfu | Lefkada | Cephalonia | Chania |
|-------------------------------|--------|--------|-----------|-------------------|--------|-----|-----------|--------|----------|------------|--------|-------|---------|------------|--------|
| Orosius orientalis            |        |        |           |                    |        |     |           |        |          |            |        |       |         |            |        |
| Phlepsius intricatus         | x      |        |           |                    |        |     |           |        |          |            |        |       |         |            |        |
| Phycotettix truncatipennis   | x      |        |           |                    |        |     |           |        |          |            |        |       |         |            |        |
| Psamotettix alienus           | x      | x      | x         | x                 | x      | x  |           |        |          |            |        |       |         |            |        |
| Psamotettix spp.             |        | x      | x         | x                 | x      | x  |           |        |          |            |        |       |         |            |        |
| Recilia schmidtgeni          | x      |        |           |                    |        |     |           |        |          |            |        |       |         |            |        |
| Selenocephalus sp.           | x      |        |           |                    |        |     |           |        |          |            |        |       |         |            |        |
| Synophropsis lauri           | x      | x      | x         | x                 | x      | x  |           |        |          |            |        |       |         |            |        |
| Thamnotettix zelleri         | x      | x      |           |                    |        |     |           |        |          |            |        |       |         |            |        |
| **Subfamily: Hecalinae**     |        |        |           |                    |        |     |           |        |          |            |        |       |         |            |        |
| Hecalus glaucescens          | x      |        |           |                    |        |     |           |        |          |            |        |       |         |            |        |
| **Subfamily: Typhlocybinae**  |        |        |           |                    |        |     |           |        |          |            |        |       |         |            |        |
| Arboridia adanae             | x      |        |           |                    |        |     |           |        |          |            |        |       |         |            |        |
| Empoasca decipiens           | x      |        |           |                    |        |     |           |        |          |            |        |       |         |            |        |
| Empoasca vitis               | x      |        |           |                    |        |     |           |        |          |            |        |       |         |            |        |
| Eupteryx stachydearum        | x      |        |           |                    |        |     |           |        |          |            |        |       |         |            |        |
| Hauptidia provincialis       | x      |        |           |                    |        |     |           |        |          |            |        |       |         |            |        |
| Zygina rhamni                | x      |        |           |                    |        |     |           |        |          |            |        |       |         |            |        |
| Zyginitia pullula            | x      | x      |           |                    |        | x  |           |        |          |            |        |       |         |            |        |
| Zyginitia sp.                | x      | x      |           |                    |        | x  |           |        |          |            |        |       |         |            |        |
| **Family: Cicadidae**        |        |        |           |                    |        |     |           |        |          |            |        |       |         |            |        |
| Cicada orni                  | x      |        |           |                    |        |     |           |        |          |            |        |       |         |            |        |
| Cicadetta montana            | x      | x      |           |                    |        |     |           |        |          |            |        |       |         |            |        |

**Abbreviation:** x, presence of insect.
were also observed on olive trees’ foliage. Nevertheless, only a small number of *N. campestris* individuals were captured on olive foliage. The small proportion of insects found on olive foliage may be due to *N. campestris* preference to grasses as feeding host plants (Ben Moussa et al., 2016).

*P. signatus*, *L. coleoptrata*, *A. alni* and *C. sanguinolenta* were recorded sporadically and in a few sampling areas. For those species, few individuals were captured. *C. sanguinolenta* and *L. coleoptrata* were captured only on ground vegetation, while the other two species were recorded both on ground vegetation and on olive trees’ foliage. Our results on the spittlebugs fauna are quite similar with those reported from other Mediterranean countries. *P. spumarius* and *N. campestris* were the most common spittlebugs of various olive orchards in Spain and Italy (Ben Moussa et al., 2016; Cornara, Saponari, et al., 2017; Dongiovanni et al., 2019; Morente et al., 2018). *L. coleoptrata* and *Cercopis intermedia* were occasionally found in Spain on ground vegetation (Morente et al., 2018). In Italy, in the region of Apulia, *C. sanguinolenta* was captured in olive groves (Ben Moussa et al., 2016) and on weeds bordering olive orchards (Cornara, Saponari, et al., 2017). All the spittlebugs found on the Greek olive groves are xylem-fluid feeders and are considered as potential vectors of *X. fastidiosa* (EFSA, 2013, 2015; Purcell, 1989).

Cicadellidae was the most abundant family of the infraorder Cicadomorpha. During the surveys, no species from the subfamily Cicadellinae, which are considered as potential vectors of the bacterium, was observed. Most Cicadellidae species were recorded mainly on the ground vegetation under the olive trees. There were only a few leafhopper species that frequently occurred on olive foliage, and all of them belonged to the subfamily Deltocephalinae with *Synophropsis lauri* and *Anoplotettix fuscovenosus* being most common. On ground vegetation, the most frequently observed species also belonged to this subfamily. In particular, they were *Psamotettix alienus*, *Exitianus capicola*, *Cicadulina bipunctata* and *Euscelis lineolatus*. *Anaceratagallia ribauti* was the most common species of the subfamily Agallinae, while the species of the subfamilies Aphrodinae and Hecalinae were seldom captured. Typhlocybinae is the second largest leafhopper subfamily after Deltocephalinae (Dietrich, 2013). However, in olive groves there were few records of those species. Typhlocybinae were observed almost exclusively on ground vegetation, and the most common species were *Empoasca decipiens* and *Zyginidia pullula*. Data about the Cicadellidae species in olive orchards of European countries are scarce. Ben Moussa et al. (2016) reported that the Cicadellidae species in Apulia belonged to the subfamily Deltocephalinae, and the most abundant species were *Thamnotettix zelleri*, *E. lineolatus*, *Anoplotettix putoni* and *S. lauri*. The Deltocephalinae fauna in Apulia was similar to Greek olive orchards. The species, found on the Greek orchards, of the subfamilies Deltocephalinae, Agallinae, Aphrodinae, Hecalinae and Typhlocybinae are all phloem feeders. According to Elbeaino et al. (2014), *E. lineolatus* and all phloem feeders, in general, can occasionally be in contact with xylem vessels and may acquire the bacterium. However, the transmission of *X. fastidiosa* by phloem fluid-feeding insects has not been demonstrated as they cannot successfully inoculate the bacterium into xylem vessels (Ben Moussa et al., 2016). Thus, no phloem feeder is considered as potential vector of the pathogen. As a result, all the leafhopper species that were observed in the surveyed olive groves do not pose a threat to the olive trees regarding the transmission of *X. fastidiosa*, as they are not considered to be vectors of the bacterium. Nevertheless, it must be mentioned that many leafhoppers are important vectors of serious diseases of many other crops. For instance, *P. alienus* is the vector of wheat.
neighbouring countries must keep plant health authorities and farm- 

*X. fastidiosa* not only in Greece but throughout Southern Europe. Regarding the molecular analysis, according to EPPO (2018), samples should be considered as ‘samples with *X. fastidiosa* detected’ when at least two screening tests are positive based on the amplification of different parts of the genome. The results of the present study revealed the absence of the bacterium in the different species of spittlebugs analysed, as none of the individuals tested for both genes was found to be positive to the bacterium. Hitherto, there is no any record of the presence of the bacterium in any host plant or insect vector in Greece. However, the presence of *X. fastidiosa* in neighbouring countries must keep plant health authorities and farmers in vigilance. The main entry pathway of *X. fastidiosa* in a country is the trade and the movement of plants for planting and the movement of infective insect vectors (EFSA, 2013). Thus, the possibility of the introduction of the bacterium in Greece in the future cannot be excluded. Further investigation is required in order to gain a better knowledge about the life cycle, seasonal appearance, feeding needs, plants most preferable for oviposition, nymph development, etc., of those two spittlebugs. In general, the robust understanding of the biology and ecology of those insects in the climatic conditions of Greece is essential in order to avoid environmentally unacceptable control methods (e.g. repeated insecticide applications) and to be able to establish an efficient control strategy in case of an outbreak of the bacterium in the country.

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**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

**AUTHORS’ CONTRIBUTIONS**

PM and DPP conceived research and secured funding. All authors conducted field samplings. SA and ICL identified the insects. DEK and VIE conducted the molecular analysis. SA, DEK and VIE wrote the manuscript. All authors commented on the manuscript. All authors read and approved the manuscript.

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are openly available online in the supporting information section of the article.

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Wang, 2010) while leafhoppers of the genus Cicadulina are vectors of pathogens of maize, cereals and sugarcane (Rose, 1978; Webb, 1987). *Macrostelles quadripectinatus* and *A. ribauti* have been reported as vectors of *Candidatus Phytoplasma solani* (Battle, Altabella, Sabate, & Laviña, 2008; Riedle-Bauer, Sara, & Regner, 2008) which infects a wide range of wild and cultivated plants, while *Fieberiella florii* is a vector of *Candidatus Phytoplasma mali* which represents one of the most economically important threats to apple trees (Tedeschi & Alma, 2006). Furthermore, *Arboridia adanae* and *Zygina rhamni* are considered serious grape pests in Eastern Mediterranean regions and in Europe (Olivier et al., 2012) while various leafhoppers are vectors of phytoplasmas in *Rubus* species (Linck & Reineke, 2019). Lastly, it is worth stating that the species *Anaconura acuticeps* of the subfamily Deltocephalinae and the species *A. adanae* and *Z. rhamni* of the subfamily Typhlocybinae are recorded for the first time in Greece.

Cicadas are also xylem-fluid feeders, but their role in transmitting *X. fastidiosa* is poorly understood (EFSA, 2013). In the surveys of the current study, the presence of cicadas was perceived in most of the olive groves during summer by their characteristic sound. However, only a few individuals were captured in the canopy of olive trees in three olive groves during the surveys. Two species of the family Cicadidae were identified as *Cicada orni* and *Cicadetta montana*. As it has been mentioned, the results about cicadas are only indicative.

Summarizing the results of our surveys, it is obvious that the main potential vectors of *X. fastidiosa* in Greek olive orchards are *P. spumarius* and *N. campestris*. Those species have a wide distribution over the country, they are present for a long period during the year, and they inhabit both the ground vegetation and the olive trees’ canopy. Those two species are also considered to be the main vectors of *X. fastidiosa* in Italy (Cornara, Cavalieri, et al., 2017; Cornara, Saponari, et al., 2017; Saponari et al., 2014) and in the Iberian Peninsula (Morente et al., 2018). Taking into account the results of all the aforementioned studies, it seems that *P. spumarius* and *N. campestris* are the most common spittlebugs of olive groves not only in Greece but throughout Southern Europe.

Regarding the molecular analysis, according to EPPO (2018), samples should be considered as ‘samples with *X. fastidiosa* detected’ when at least two screening tests are positive based on the amplification of different parts of the genome. The results of the present study revealed the absence of the bacterium in the different species of spittlebugs analysed, as none of the individuals tested for both genes was found to be positive to the bacterium. Hitherto, there is no any record of the presence of the bacterium in any host plant or insect vector in Greece. However, the presence of *X. fastidiosa* in neighbouring countries must keep plant health authorities and farmers in vigilance. The main entry pathway of *X. fastidiosa* in a country is the trade and the movement of plants for planting and the movement of infective insect vectors (EFSA, 2013). Thus, the possibility of the introduction of the bacterium in Greece in the future cannot be excluded. Further investigation is required in order to gain a better
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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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