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Philaenus italosignus a potential vector of *Xylella fastidiosa*: occurrence of the spittlebug on olive trees in Tuscany (Italy)

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Abstract

The spittlebug *Philaenus spumarius* (L.) is considered the main vector of *Xylella fastidiosa* in Apulia (Italy), however, another species of the same genus is present in Italy: *Philaenus italosignus* Drosopoulos et Remane. Recently its competence in acquiring the bacterium from infected olives and transmitting it to other plants was verified in the laboratory. The spittlebug seems to be confined to areas where its larval host plant (*Asphodelus ramosus* L.) is present, though no precise data on its distribution are available. This study confirms the occurrence of *P. italosignus* in Tuscany, where it was found on *A. ramosus* (at high population densities) and also on olive trees.

Key words: olive quick decline syndrome, host range.

Introduction

The genus *Philaenus* (Rhynchota Aphrophoridae) includes eight species, which have recently been sorted in two taxonomical groups: the "*spumarius*" group and the "*signatus*" group (Drosopoulos and Remane, 2000; Remane and Drosopoulos, 2001). The first group "*spumarius*" consists of *Philaenus spumarius* (L.), *Philaenus tesselatus* Melichar, *Philaenus loukasi* Drosopoulos et Asche and *Philaenus arslani* Abdul-Nour et Lahoud. The second group "*signatus*" includes four species: *Philaenus signatus* Melichar, *Philaenus italosignus* Drosopoulos et Remane, *Philaenus maghresignus* Drosopoulos et Remane, and *Philaenus tarifa* Remane et Drosopoulos. Indeed, results of phylogenetic analyses carried out on two mtDNA gene fragments, well support the existence of the "*spumarius*" and "*signatus*" groups of species, with the exception of *P. maghresignus* that ought to be considered sister taxon to the two clades (Maryańska-Nadachowska *et al*., 2010).

Among these eight spittlebugs, the Holarctic *P. spumarius*, is the most widely distributed species, also with the widest range of host plants both at adult and nymph stages. The species belonging to the "*signatus*" group are restricted to the Mediterranean area. They are larger if compared with *P. spumarius* (Drosopoulos and Remane, 2000, Maryańska-Nadachowska *et al*., 2010), and lastly, they have only one ascertained host plant during larval development, *i.e.* the lily *Asphodelus ramosus* L. = *aestivus* = *microcarpus* (Drosopoulos, 2003; Maryańska-Nadachowska *et al*., 2012). These four species are sympatric with *P. spumarius*, while they are in part allopatric with each other, being *P. spumarius* and *P. italosignus* the only species present in Italy (Maryańska-Nadachowska *et al*., 2010). While *P. spumarius* is widely distributed in Italy, *P. italosignus* seems to be confined to areas where its host plant is present, even though no precise data are available. This latter species was first described by Drosopoulos and Remane (2000) based on genitalia morphological characters of specimens from southern Italy (Lazio, Apulia, and Sicily Regions). During later studies *P. italosignus* adults were collected during summer from forests with *Quercus ilex* L. and *Acer campestre* L. in Latium (Guglielmino *et al*., 2005) and during spring from *A. ramosus* in Sicily (Drosopoulos, 2003; Maryańska-Nadachowska *et al*., 2008; 2010; 2012).

Recently, spittlebugs acquired importance because of their role as vectors of an olive disease detected for the first time in Europe (in Apulia, Italy) in 2013 (Saponari *et al*., 2013), the olive quick decline syndrome (OQDS), caused by *Xylella fastidiosa* subsp. *pauca* ST53 (Wells *et al*., 1987). *P. spumarius* is considered the main vector of *X. fastidiosa* in Apulia (Saponari *et al*., 2019), however, Cavalieri *et al*. (2018) showed, under experimental conditions, the transmission capabilities of two additional spittlebugs: *P. italosignus* and *Neophilaenus campestris* (Fallen). More specifically, the authors demonstrated their competence in acquiring the bacterium from infected olives and transmitting it to other plants. Anyway, these spittlebugs are considered of minor importance in spreading the disease in Apulia, because in the *X. fastidiosa* infected areas *N. campestris* was recorded at low populations densities (and seldom on olive canopy), while *P. italosignus* was never recorded (Saponari *et al*., 2019). Recently the situation in Italy has become even more complicated because of the recent record of *X. fastidiosa* subsp. *multiplex* in Tuscany (Grosseto Province, Italy) (Marchi *et al*., 2018).

Materials and methods

Based on this new finding, from February 11th to June 1st 2019 we surveyed an area in the Maremma Regional Park (Grosseto Province, Italy) (42°40'13"N 11°05'10"E), located about 30 km north from the infected site, to investigate the presence of xylem sap-feeders. Since *A. ramosus* is common in the park (Selvi, 2010), and moreover it represents, in some local olive groves, almost the entire herbaceous layer, we paid particular attention in verifying the occurrence of *P. italosignus*. In fact, new data about its distribution range and foodplants would allow to better address the potential role of this species in the spreading of OQDS. Surveys were carried out by collecting spittlebug adults with a sweeping net or an aspirator on the herbaceous vegetation (including the lily) and on olives. In the laboratory, adults of *Philaenus* spp. were identified by morphological analysis and kept separately. Total body length (from the top of the head to the end of the fore wings) of each specimen was recorded to compare our data with those by Drosopoulos and Remane (2000). To define the size range of *P. italosignus* females only specimens collected directly on the lily were considered, thus limiting the risk of including other *Philaenus* species. Then, males were dissected for the analysis of the genitalia. To this purpose, the last abdominal segments were taken from the specimens, then they were treated with 10% KOH for 15 minutes at boiling temperature. Finally, genitalia were extracted and compared with the work by Drosopoulos and Remane (2000).

To further confirm species identification, molecular analyses were carried out using 2 adults (one female, M4, and one male, M11) collected from the lily. The DNA was extracted following the CTAB protocol described in Marzachì *et al.* (1998). A fragment of the cytochrome c oxidase I (COI) gene, was amplified using primers LEP-F1 5'-ATTCAACCAATCATAAAGATAT'-3 (Hebert *et al.*, 2004) and LEP-R1 5' -TAAACTTCTGGATGTCCAAAAAATCA-3' (Gwiazdowski *et al.*, 2015) and the following reaction mixture: 1 DreamTaq Green Buffer (Thermo Scientific), 1 U DreamTaq polymerase (Thermo Scientific), 0.2 mM each dNTP (Thermo Scientific), 0.2 µM each primer, approximately 20 ng template DNA and DNase free water to a final volume of 50 µL. Thermal cycling consisted of 3 minutes at 95 °C for initial denaturation; 40 cycles of denaturation at 95 °C for 30 seconds, annealing at 51 °C for 60 seconds, extension at 72 °C for 30 seconds; followed by 5 minutes at 72 °C for final extension. PCR products obtained were visualized after electrophoresis in 1% agarose gels in 1 × Tris-acetate-EDTA (TAE) buffer and staining with ethidium bromide (0.5 µg mL^{-1}). Amplicons were purified using ExoSAP-IT (USB Corp.) according to the manufacturer's protocol and direct sequencing was carried out in both directions. Nucleotide sequences were visualized using CHROMAS LITE v. 2.01 (Technelysium) and aligned using MUSCLE as implemented in MEGA 7 (Kumar *et al.*, 2016). Identity searches of the resulting consensus sequences were performed on the INSDC database (http://www.insdc.org/).

Results and discussion

In the study site a total of 123 adult males of *Philaenus* spp. were collected. From morphological analysis 120 were assigned to *P. italosignus*, while three were *P. spumarius* (table 1). Based on body size range indicated by Drosopoulos and Remane (2000), 111 adult females were identified as *P. italosignus* and seven as *P. spumarius*. Size ranges of our samples resulted quite similar to those by Drosopoulos and Remane (2000). In fact, as regards *P. italosignus* females these authors recorded an almost identical size range (7.03-8.13 mm) to that of females we collected from the lily (table 2). On the other hand, they indicated a slightly lower size range for males: 6.4-7.3 mm instead of 6.6-7.7 mm (table 2).

Table 1. Adults of *P. italosignus* and *P. spumarius* caught in the Maremma Regional Park (Tuscany, Italy) from various host plants.

Host plants	*P. italosignus*			*P. spumarius*			*Philaenus* spp.
	Males	Females	Total	Males	Females	Total	
Lily	106	102	208	0	0	0	208
Olive	9	8	17	1	5	6	23
Other weeds	5	1	6	2	2	4	10
Total	120	111	231	3	7	10	241

Table 2. *P. italosignus* specimens from the Maremma Regional Park (Tuscany, Italy).

	N° of specimens analysed	Total body length (mm)	
		Mean ± SD	Range
Males	120	7.26 ± 0.24	6.6-7.7
Females*	83	7.72 ± 0.20	7.1-8.1

*only females collected directly on lily with the aspirator were considered.

Molecular analysis confirmed morphological identification of *P. italosignus.* When the identical 660 bp nucleotide sequences we obtained from the COI gene of specimens M4 and M11 were trimmed to 399 bp for comparison purposes, the identity with the COI gene fragment of *P. italosignus* (FJ516388), was 100%. Identities with the homologous region of the other members of the "*signatus*" group of species, *P. tarifa* (FJ516389) and *P. signatus* (FJ5163890), were 96 and 93%, respectively (Maryańska-Nadachowska *et al*., 2010).

P. italosignus resulted the only species of the genus found among specimens collected directly on the lily. However, adults were also caught on olives and other herbaceous plants. A total of 208 *P. italosignus* adults were collected on lily from April 8th to June 1st (table 1). Starting from May 8th, 10 *Philaenus* spp. adults were caught on other herbaceous plants and 23 from olive trees (table 1). Both species were found on other weeds, and although in little numbers, they were rather balanced (table 1). As regards the olive, 17 specimens were *P. italosignus* (nine males and eight females) and six specimens (one male and five females) *P. spumarius*. These preliminary data show that in environments where the lily is particularly abundant *P. italosignus* may occur on olive more frequently than *P. spumarius*. However, more surveys are needed to better address the abundance of both species on olive during the whole activity period of the two spittlebugs. Indeed, in May all sampled *P. italosignus* specimens were adults, while we still observed many *P. spumarius* nymphs. Therefore, later surveys could provide different results.

Conclusions

This study confirms the occurrence of *P. italosignus*, one of the potential vectors of *X. fastidiosa*, in Tuscany. Although this spittlebug is considered of minor importance in spreading the bacterium in Apulia infected areas (Saponari *et al*., 2019), it could have a more important role in other areas. In our study site, 30 km north from the recent record of *X. fastidiosa* in Tuscany, the spittlebug was found at high population densities on the lily; in addition, in the month of May it was already found also on olive trees. During these surveys other spittlebugs were also found besides *P. italosignus* and *P. spumarius*, for example *N. campestris*; anyway, further details about their occurrence on olive trees in the study area will be reported in following papers since the study is still in progress.

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