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ORIGINAL ARTICLE

# First record of *Aleurocanthus camelliae* Kanmiya & Kasai, 2011 (Hemiptera, Aleyrodidae) from Italy, on ornamental *Camellia* spp. plants

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**Abstract**

This paper provides a first report of *Aleurocanthus camelliae*, the Camellia spiny whitefly, from Italy. The pest was found on plants of *Camellia* spp. grown in the nursery. Brief morphological and biological information is provided on this whitefly, as well as some considerations on the phytosanitary measures to be adopted to reduce the potential risk of its spread on ornamental plants in Europe and the EPPO region.

**Premier signalement d'*Aleurocanthus camelliae* Kanmiya & Kasai, 2011 (Hemiptera, Aleyrodidae) en Italie, sur des plants ornementaux de *Camellia* spp.**

Cet article présente le premier signalement de l'aleurode *Aleurocanthus camelliae* en Italie. Cet organisme nuisible a été signalé sur des plants de *Camellia* spp. cultivés en pépinière. Cet article présente brièvement des informations sur la morphologie et la biologie de cet aleurode, ainsi que des considérations sur les mesures phytosanitaires à adopter pour réduire son risque de dissémination sur plantes ornementales en Europe et dans la région OEPP.

**Первое обнаружение *Aleurocanthus camelliae* Kanmiya & Kasai, 2011 (Hemiptera, Aleyrodidae) в Италии на декоративных растениях *Camellia* spp.**

В данной работе представлено первое сообщение о щетинистой камелиевой белокрылке *Aleurocanthus camelliae*, из Италии. Вредный организм был найден на растениях *Camellia* spp., выращиваемых в питомнике. По этой белокрылке представлена краткая морфологическая и биологическая информация, а также некоторые соображения по фитосанитарным мерам, которые должны быть приняты для снижения потенциального риска ее распространения на декоративные растения в Европе и регионе ЕОКЗР.

## 1 | INTRODUCTION

The insect group of Aleyrodidae (commonly known as whiteflies due to the white powdery wax secretion covering the entire body and wings of the adults) includes more than 1500 species worldwide (Mound & Halsey, 1978; Martin & Mound, 2007), mostly having an intertropical distribution but with various species extending also to temperate regions. They all exclusively feed on plants, causing both direct (suction of phloem sap and production of honeydew, on which sooty mould develops) and indirect (virus transmission) damage (Martin *et al.,* 2000). Crop losses caused by whiteflies and whitefly transmitted virus diseases are difficult to estimate because of the vast number of crops they attack and their diverse distribution. In particular, quarantine importance of whiteflies in the EPPO region is worth a special mention due to the current global climate change, the present world distribution of several whitefly pests and their host plants, as well as the increasing plant trade, which increases the risk of introduction of noxious whitefly pests.

Concerning movement in trade, whiteflies are among the insect groups with the highest number of species accidentally introduced into Italy during the last decades (Rapisarda *et al.,* 2017). Among these recent invasions, the record of *Aleurocanthus spiniferus* (Quaintance) on citrus in southern Italian areas (Porcelli, 2008) is noteworthy due to potential risk of spread of this species in citrus crops in the country (Rapisarda & Longo, 2021). The genus *Aleurocanthus* (Quanitance & Baker) presently includes around 80 species worldwide (Kanmiya *et al.,* 2011; Dubey & Ko, 2012; Gillespie, 2012; Jansen & Porcelli, 2018), but *A. spiniferus* was the only one known prior to this report for the Italian fauna.

In September 2020, the presence of a whitefly species was noted on nursery-grown plants of the genus *Camellia* L. (Ericales, Theaceae); no whiteflies were recorded before in Italy on this plant genus. The morphology of both adults and nymphs of this whitefly allowed it to be ascribed to the genus *Aleurocanthus*; however, some characters raised the suspicion that it was a different species from *A. spiniferus*, therefore a possible new introduction in Italy. This hypothesis has been confirmed by morphological and molecular study of this whitefly material, which led to its identification as *A. camelliae* Kanmiya & Kasai, also known as the Camellia spiny whitefly.

## 2 | MATERIAL EXAMINED

Adults and nymphs of this whitefly were observed on the lower surface of the leaves in eight plant nurseries in the Pistoia province (Tuscany, Italy) where *Camellia sasanqua* Thunb. plants were grown outdoors in plastic containers. The plants inspected generally had sooty mould on the leaves although the whitefly infestation was generally scarce. From the infested leaves collected in each nursery, adults and nymphs of the whitefly were collected and preserved in ethanol for subsequent laboratory investigations.

## 3 | DIAGNOSIS

The material collected was identified both on a morphological basis at the Department of Agriculture, Food and Environment (D*i*3A) of the University of Catania and through molecular analysis at the Laboratory of Phytopathological Diagnostics and Molecular Biology, Plant Protection Service of Tuscany.

### 3.1 | Treatment of specimens for morphological identification

According to the established practice in whitefly taxonomy, morphological identification was based on characters of the fourth-instar nymphs (also known as puparia). About 50 fourth-instar nymphs were cleaned of wax through a treatment in 95% ethanol with gentle heating, then treated with cold 10% solution of KOH for several days until the black cuticle was bleached and specimens were a light brown colour. Bleached nymphal material was rinsed in 70% ethanol with gentle heating for 3 min, placed in glacial acetic acid and subsequently in clove oil for about 15 min each, and finally slide mounted in Canada balsam. Morphological observations and measurements on the slide-mounted specimens were carried out using a phase contrast microscope (Olympus BX51TF, equipped with a digital camera Olympus Camedia C30-30 zoom).

### 3.2 | Methods for molecular analysis

DNA was extracted from 10 fourth-instar nymphs and 10 adults collected from infested plant leaves according to the methodology described for other quarantine pests by Rizzo *et al.* (2020a,b). DNA extraction was carried out on two pooled samples (each consisting of five fourth-instar nymphs and five adults), from which five technical replicates were extracted for each stage of development (fourth-instar nymphs and adults). The quality and quantity of extracted DNA were analysed using a QiaExpert spectrophotometer (Qiagen, Hilden, Germany). DNA was eluted in 100 μL of nuclease-free water and used immediately in qPCR or stored at −20°C until use. In addition, to quantitatively assess the extracted DNA and its suitability for qPCR tests, the DNA samples were first amplified using 18S uni-F/18S uni-R primers (5′-GCAAGGCTGAAACTTAAAGGAA-3′/5′-CCACCACCCATAGAATCAAGA-3′) and the uni-P

18S probe (HEX-ACGGAAGGGCACCACCAGGAGT-BHQ1) targeting the highly conserved region on 18S ribosomal DNA (Ioos *et al.,* 2009).

A specific SybrGreen qPCR test was designed for the diagnosis of *A. camelliae*. The primer pairs were designed by OligoArchitectTM Primers and Probe Design Online software (Sigma-Aldrich, St Louis, USA) on the ATP6 synthase mitochondrial gene (Fig. 1 and Table 1). The primer design considered the primer melting temperatures, the amplicon length and the absence of secondary structures.

The primer specificity was analysed by the software BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi), searching for the most closely related nucleotide sequences and aligning them using the MAFFT software package (Katoh and Standley, 2013) implemented within the software Geneious® 10.2.6 (Biomatters, http://www.geneious.com). The results are shown in Fig. 2.

The unrooted phylogenetic tree of *A. camelliae* was created using the software Geneious 10.2.4, according to the neighbour-joining method and the Tamura-Nei model, with 1000 bootstrap replicates (Fig. 3).

CACTTAAATTTTAATAAGTCTTTTTGAAGTTTACGACCCTTACACGGCCATTCTTGGACTAAGTCTTAATTGAGTTTTTG
TTTTTTTTTTTTTTTTTTTTTTTTTTTGGAGGGTATTGATGTGCTATAAGTATCTGAAGTTTAACTGAGTTTTACTTAGTT
CGAGGATTGTTGAGTGAATTTAAAAATTTTTTTTCTGTCATGAAGAGTGAGTTTGGTTTATTAATATTTATTTGTCTTTT
TGTTTACATTTTGTTTGTAAATTTGTTGGGATTAGTTCCTTACACTTTTGCCTGTTCTGCTCATTTTGTGTTTGCACTTA
GTCTTGGATTACCAATATGACTGGGTTTTATATTGCTTGGCTGGTTTAATTTTACTAATTCTATATTTTGTCATTTGATT
CCGGTTGGAACTCCTATAGTACTTGTTTCATTTATGGTAATTATTGAAACTATCAGGAATTTAATTCGTCCCTGGTCTCT
GAGTATTCGGCTTATGGCTAATATAATTTCTGGTCATTTATTAATAAGATTGTTGGGGGACTGTGGTATTTTTTTAATTT
TTATGCAATTAGTTCTGTTTAGATTTGAATTTTTTGTTTGTTTTATTCAGTCCTATGTGTTTTCTGCATTATTAACTTTA
TATTCAAGTGAAATTTAGTTTTAACTTTTTATAGCACAGCTATAGCCCTAATTGATTGGCAATTCCATAATGAAACTATA

**FIGURE 1** Genomic region of interest from which the oligos were selected by the *OligoArchitectTM Primers and Probe Design* Online software

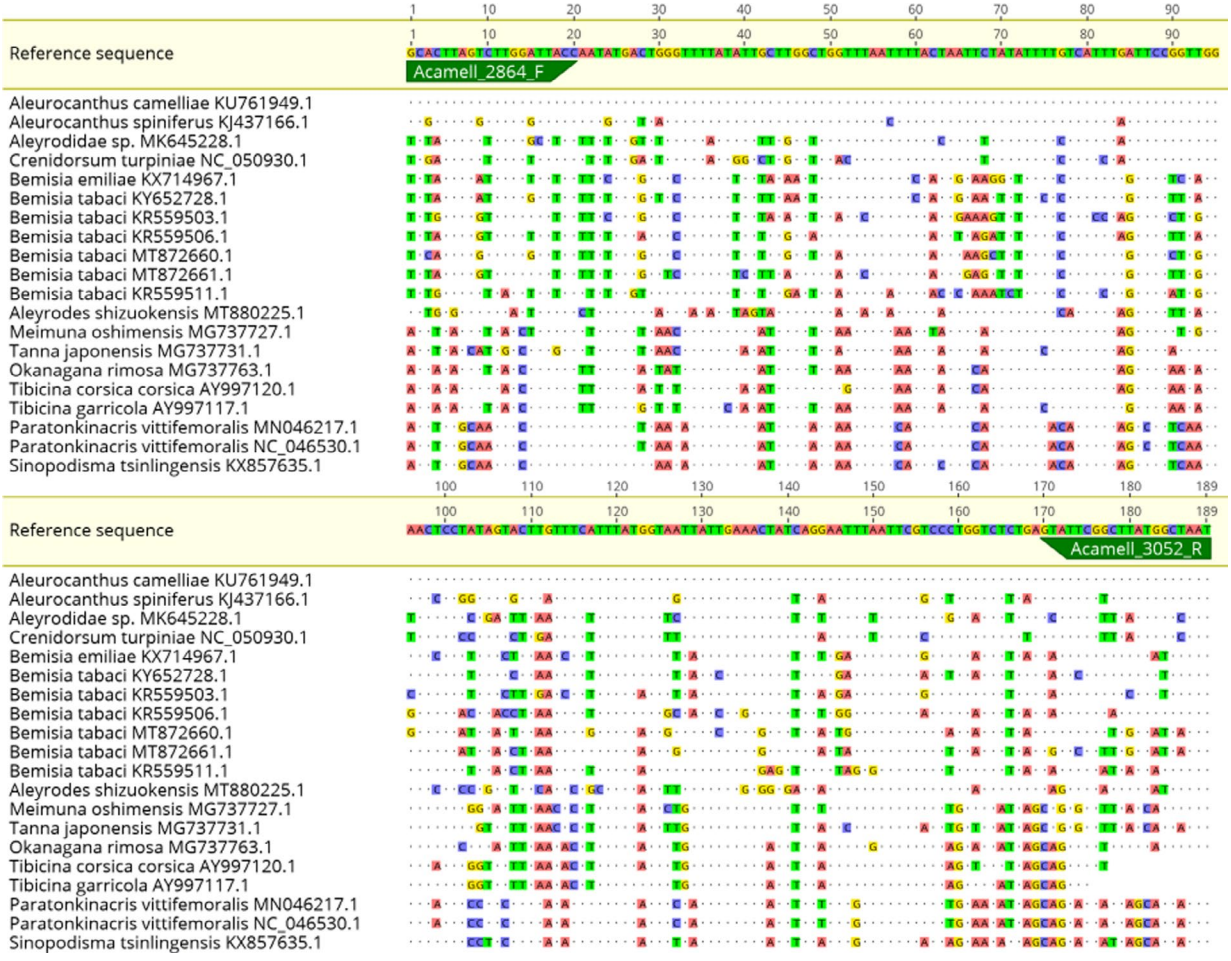


**FIGURE 2** Alignments resulting from the *in silico* theoretical SYBR Green amplicons

The reactions were carried out in a 20 μL volume containing 0.4 μM of each primer, 10 μL of SsoAdvanced Universal SYBR Green Supermix (Biorad, Hercules, CA, USA) and 10–20 ng of extracted DNA. To determine the optimal annealing temperature, a thermal gradient from 50°C to 60°C was performed. The optimal amplification reaction consisted of an initial denaturation of 2 min at 95°C followed by 40 cycles of denaturation of 10 s at 95°C and a combined annealing and elongation step at 55°C for 40 s. At the end of the amplification protocol, the test was confirmed by means of melting curve analysis from 65°C to 95°C (0.5°C increments per read with 10 s hold time). The melting peak was 78 ± 0.5°C. In each reaction, *Aleurocanthus spiniferus* and *Dialeurodes citri* were taken as nontarget references. Considering the relevant genetic proximity, in particular with *A. spiniferus*, the identification of nontarget organisms was made not only on the basis of morphology, but also by biomolecular analysis, with gene amplifications (starting from DNA extracts of adults of *A. spiniferus* and *D. citri*) in endpoints (LCO1490/HCO2198) targeting cytochrome oxidase (COI) subunit 1. The real-time reactions were performed on a CFX96 thermocycler (Biorad). Reactions were performed in duplicate including a negative control (no DNA) for each set of reactions. Three amplicons of the SYBR Green qPCR reactions for *A. camelliae* were purified with a QIAquick PCR Purification kit (Qiagen) and sequenced (CIBIACI, Florence, Italy).

## 4 | RESULTS

On microscopic examination, and based on the keys provided by Kanmiya *et al.* (2011) and by Jansen and Porcelli (2018), the fourth-instar nymphs examined (Fig. 4) showed characters corresponding to *Aleurocanthus camelliae* and falling within the morphometric range attributed to this species, with special reference to:

(i) marginal crenulations of puparium small and almost rounded, varying in number from 182 to 194
(ii) clearly defined ovoid cephalic eyespot, each one close to the base of the corresponding third submarginal spine
(iii) length of eight abdominal tergite 49.3–53.7 μm
(iv) length of vasiform orifice 84.6–86.9 μm.

The molecular analysis confirms the results from morphological study. In particular, the extracted samples gave a good result from the point of view of amplificability, in fact the mean values of Cq of the qPCR Probe (18S rDNA) that originated were equal to 18.26 ± 1.6. No

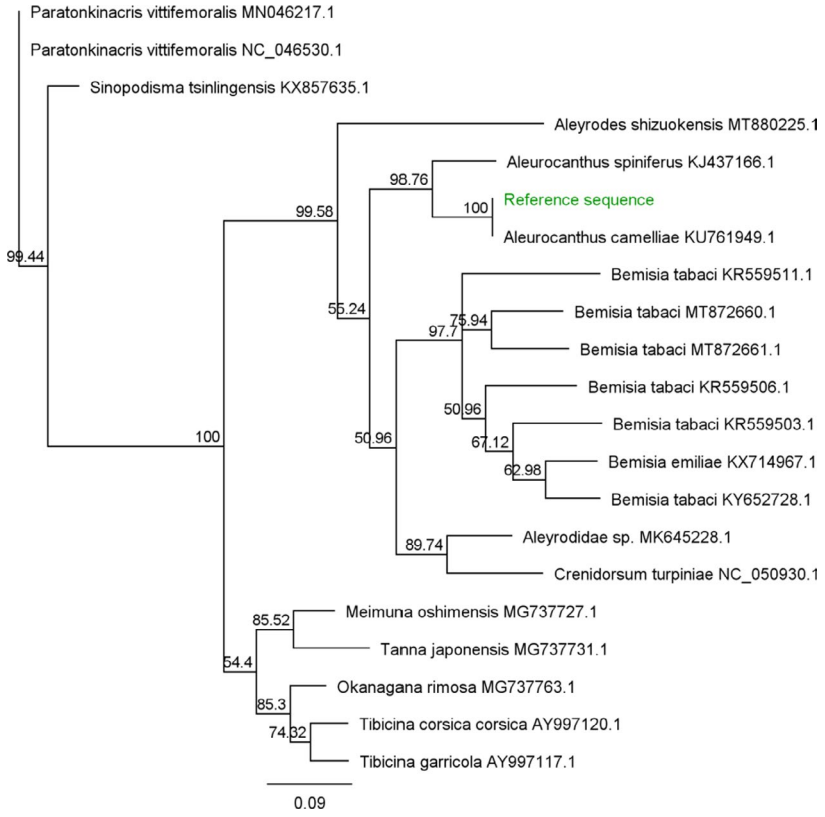


**FIGURE 3** Unrooted phylogenetic tree from GenBank sequences of *A. camelliae* and related (non-*A. camelliae*) species used in the SYBRGreen-based qPCR protocol. The phylogenetic tree was constructed using Geneious 10.2.4 according to the neighbour-joining method and the Tamura–Nei model with 1000 bootstrap replicates

amplification occurred with the nontarget species considered (Fig. 5).

The sequence analyses performed show as a result only *A. camelliae* (accession number KU761949.1) using the BLAST software (https://blast.ncbi.nlm.nih.gov/Blast.cgi) searching the most related nucleotide sequences. To support these results, a qPCR test with TaqMan Probe specific for the diagnosis of *A. camelliae* is being developed. The biomolecular investigations, as a whole, support the diagnosis of *A. camelliae* presence in the samples.

## 5 | GENERAL NOTES ON *A. CAMELLIAE*

### 5.1 | Morphology

Based on the literature (Kanmiya *et al.,* 2011; Jansen & Porcelli, 2018), the main morphological characters of this species are provided below.

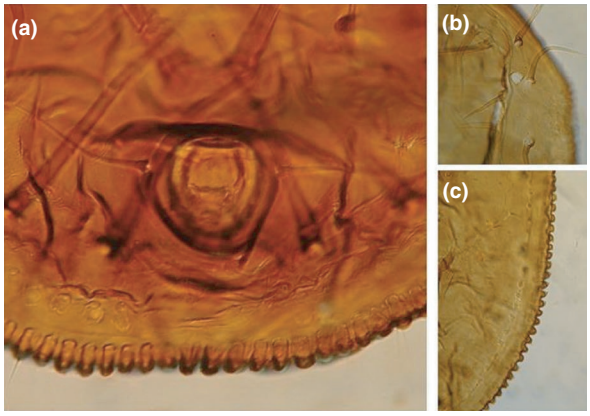


**FIGURE 4** *Aleurocanthus camelliae* Kanmiya & Kasai, slide-mounted puparia from Italy: (a) posterior part of the abdomen, dorsal view, with the vasiform orifice; (b) cephalic eyespot, close to the base of the third submarginal spine; (c) marginal crenulations

Adults of *A. camelliae* are strongly dimorphic, with males 0.90–1.10 mm and females 1.25–1.40 mm long. When mature, they are orange to brown in colour, covered by a greyish wax powder coating. Head, in dorsal view, 240–275 µm wide, with 260–320 µm long antennae and compound eyes each having its upper and lower part connected by three ommatidia. Forewings 0.84–0.90 mm (males) to 1.1–1.2 mm (females) long, dark grey in colour with nine small whitish-grey spots. Vasiform orifice almost rounded, 45–60 µm long, nearly 1.2–1.3 times as long as wide, largely closed by the 20–35 µm long operculum, which covers the 23–28 µm long lingula.

Nymphs settle on the leaf underside; they are flattened, oval in shape, gradually darkening after egg hatching to become black, with a white marginal waxy fringe. The fourth-instar nymph (puparium) is 0.65–0.86 mm (males) to 0.98–1.24 mm (females) long and 0.39–0.57 mm (males) to 0.62–0.86 mm (females) wide. Margin crenulated, with 6–10 teeth/100 µm (total number of marginal teeth: 158–196). Dorsum with long glandular spines arranged as follows: 10–11 pairs along the submargin; 16 pairs on the subdorsum, of which nine pairs in the cephalothorax (arranged in two lines, one external with five pairs and one internal with four pairs) and seven pairs in the abdomen (arranged in a single row); three pairs on the submedian part of abdominal segments first to third. Cephalic eyespots clearly visible, ovoid in shape, each one located close to the base of the third submarginal spine. Abdominal tergite eighth 42–56 µm long. Vasiform orifice located on an almost elevated cone, 80–88 µm long and 63–68 µm wide, almost entirely covered by the operculum, which conceals the lingula.

### 5.2 | Geographical distribution

*A. camelliae* is probably native to China; and from here it was likely introduced to Japan, where it was described (Kanmiya *et al.,* 2011; Saito *et al.,* 2012),

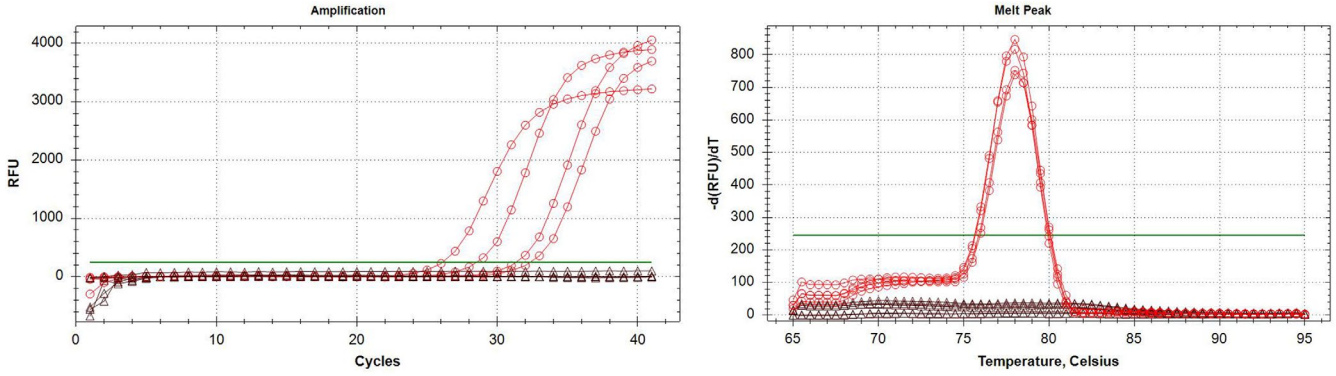


**FIGURE 5** Amplification curves and melting peaks for *A. camelliae* (circles, *A. camelliae*; triangles, *A. spiniferus* and *D. citri*)

**TABLE 1** List of the primers used in SYBR Green assay

| Primer name | Length (bases) | Sequence 5′–3′ | Nucleotide position | Product size (bp) | Reference sequence |
|---|---|---|---|---|---|
| Acamell_2864_F | 20 | GCACTTAGTCTTGGATTACC | 2864–2884 | 189 | KU761949.1 |
| Acamell_3052_R | 20 | ATTAGCCATAAGCCGAATAC | 3052–3032 | | |

and to Indonesia (Samsu Adi & Susanti, 2020). Apart from these East Asian countries, *A. camelliae* has been recorded till now only in the Netherlands (Jansen & Porcelli, 2018), especially as interceptions on camellia plants imported from China or Japan but also on potted camellia plants grown in a few localities scattered over the country. The present report, which is the first one from Italy, allows us to hypothesize a wider diffusion of the insect than is currently known, suggesting that special care is required for phytosanitary controls of imported material.

### 5.3 | Host plants and life cycle

According to the scarce literature available to date (Kanmiya *et al.,* 2011; Yamashita *et al.,* 2016), *A. camelliae* lives on plants of the genera *Camellia* L. (Theaceae): *C. japonica* L., *C. sasanqua* Thunb. and *C. sinensis* (L.) Kuntze (also known as the tea plant), *Cleyera* Thunb. (Theaceae) (*C. japonica* Thunb.), *Eurya* Thunb. (Theaceae) (*E. japonica* Thunb.), *Illicium* L. (Illiciaceae) (*I. anisatum* L.) and *Zanthoxylum* L. (Rutaceae) [*Z. piperitum* (L.) DC]. All presently known host plants of this whitefly are native to East Asia, and this highlights the importance of the pathway of movement via trade of its host plants, many of which are of significant ornamental interest, or to the widening of *Camellia sinensis* cultivation in new areas for tea production.

The biology and life cycle of this insect are poorly known. It is reported to have two to five generations per year in Japan (Kasai *et al.,* 2012). Vibratory signals emitted by males to attract females have been studied by Kanmiya *et al.* (2011).

### 5.4 | Damage to plants

Negative effects by *A. camelliae* are reported from East Asia on tea plants, *Camellia sinensis* (Uesugi *et al.,* 2016; Yamashita *et al.,* 2016), to which the whitefly may cause vigour reduction and leaf quality degradation. In tea-producing areas, secondary effects of heavy infestations by this whitefly are also reported by farmers due to the unpleasant inhalation of flying adults during field work and harvesting operations (Kasai *et al.,* 2012).

On the contrary, no particular damage has been noted till now in all cases when this insect has been found in Europe (in the Netherlands on ornamental plants) (Jansen & Porcelli, 2018). In the Italian finding here reported, the species has been detected at low population density, so that the consequent reduced levels of toxic saliva injection, sap suction and honeydew production did not cause any significant damage to the detected infested plants.

## 6 | PEST AND QUARANTINE IMPORTANCE

*A. camelliae* is an important pest, especially in tea cultivations of East Asia. In new European environments where it has been recently detected, the potential risk represented by this insect for ornamental exotic Theaceae should be assessed to define any phytosanitary measures to be adopted.

As for all whiteflies, adults of *A. camelliae* cannot fly for long distances, thus trade and the international movement of host plants is the main pathway for spread. Appropriate care must therefore be implemented in transferring plant material on which this whitefly could develop to prevent its eventual spread and further interceptions in the EPPO region.

Among natural enemies useful for biological control of *A. camelliae*, *Encarsia smithi* (Silvestri) (Hymenoptera: Aphelinidae) is reported as an active parasitoid in East Asia, with the parasitism rate reaching up to 97% in tea cultivations (Ozawa & Uchiyama, 2013).

In the Italian finding of *A. camelliae*, most of the collected fourth-instar nymphs (puparia) were found to have been parasitized, showing the typical emergence hole of an endophagous adult parasitoid. Unfortunately, no active parasitisation was present in the material examined and this prevented its adequate identification.

## 7 | CONCLUSIONS

The pest risk analysis of *A. camelliae* is still to be carried out. However, in case of further interceptions of this species, and to reduce any risk of its spread in Europe, eradication of outbreaks, involving the use of chemical control, could be suggested in the future for the EPPO region.

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