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# An *Eretmocerus* Species, Parasitoid of *Aleurocanthus spiniferus*, Was Found in Europe: The Secret Savior of Threatened Plants

**Gianluca Melone [1], Roberta Ascolese [2,3], Francesco Nugnes [2,*], Francesco Porcelli [4], Carmelo Rapisarda [5], Alessia Farina [5], Ugo Picciotti [4], Francesca Garganese [4] and Stefania Laudonia [1,6]**

[1] Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055 Portici, Italy; gianluca.melone1998@gmail.com (G.M.); laudonia@unina.it (S.L.)
[2] National Research Council, Institute for Sustainable Plant Protection, P.le E. Fermi 1, 80055 Portici, Italy; roberta.ascolese@ipsp.cnr.it
[3] Department of Biology, University of Naples Federico II, Via Cinthia 24, 80126 Naples, Italy
[4] Department of Soil, Plant, and Food Sciences, University of Bari Aldo Moro, Via Amendola 165/a, 70126 Bari, Italy; francesco.porcelli@uniba.it (F.P.); ugo.picciotti@uniba.it (U.P.); francesca.garganese@uniba.it (F.G.)
[5] Department of Agriculture, Food and Environment, Applied Entomology Division, University of Catania, Via Santa Sofia n. 100; 95123 Catania, Italy; carmelo.rapisarda@unict.it (C.R.); alessia.farina@unict.it (A.F.)
[6] Center for Studies on Bioinspired Agro-Environmental Technology, BAT Center, University of Naples Federico II, 80055 Portici, Italy
* Correspondence: francesco.nugnes@ipsp.cnr.it; Tel.: +39-06499327826

**Abstract:** This study focuses on the first detection in Europe of a parasitoid *Eretmocerus* sp. gr. *serius* (Hymenoptera: Aphelinidae) on the Orange Spiny Whitefly, *Aleurocanthus spiniferus* (Quaintance) (Hemiptera: Aleyrodidae). Through extensive field surveys, this study investigates the occurrence of the aphelinid in several regions across central and southern Italy. Different sites were monitored to investigate the *Eretmocerus* sp. gr. *serius* parasitization rate on *A. spiniferus*, shedding light on its potential as a biological control agent against the invasive whitefly pest. The research results offer a hopeful prospect for progressing sustainable and eco-friendly methods in handling *A. spiniferus* infestations in citrus crops, highlighting the importance of natural enemies in integrated pest management programs. The research emphasizes the importance of the recently discovered parasitoid for European agriculture, paving the way for inventive approaches in pest control and cultivation practices.

**Keywords:** biological control; invasive pest; natural enemies; OSW; pest control strategies

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## 1. Introduction

The genus *Aleurocanthus* Quaintance & Baker (Hemiptera: Aleyrodidae) includes nearly 100 species worldwide, a few of which are pests of cultivated plants [1]. In addition to the A2 list, the EPPO-quarantine (European and Mediterranean Plant Protection Organization) has recommended the Orange Spiny Whitefly (OSW), *Aleurocanthus spiniferus* (Quaintance); these also include, among others, the Citrus Blackfly; the *Aleurocanthus woglumi* Ashby, often misidentified with the OSW (Jansen and Porcelli, 2018); and *Aleurocanthus citriperdus* Quaintance & Baker, the latter two not occurring in the EPPO region so far [2–4].

A further species, *Aleurocanthus camelliae* Kanmiya & Kasai, has knocked and then entered the European doors, riding on imported *Camellia* sp. bonsai, pot plants, and shrubs [5,6].

*Aleurocanthus spiniferus* is native to southeast Asia and has spread to most tropical and subtropical regions, including Africa, Australia, and the Pacific Islands [4,7]. It entered Europe in 2008, when it was first recorded in Italy, in the Province of Lecce (Apulia), on

*Citrus sinensis* (L.) Osbeck [8,9]. Since then, it has been found in several other Italian areas [3,10–13] and has spread to other Mediterranean countries [11,14–16], being presently listed as a quarantine pest for the European and Mediterranean area [3,17]. The most recent findings and interceptions also report its presence in mainland Europe (Belgium, the Czech Republic, and France) [3].

Orange Spiny Whitefly infests about 90 plant species [18], preferring *Citrus* spp. and being abundant on grapes (*Vitis vinifera* L.) [11,14–16]. Among cultivated plants, *A. spiniferus* also infests guava (*Psidium guajava* L.), pears (*Pyrus* spp.), persimmon (*Diospyros kaki* L.f.), and roses (*Rosa* spp.) [19]. As its geographical distribution increases, along with subsequent interceptions and studies, records have also increased in the range of its host plant species [11]. It has also been reported on the Tree of the Heaven, *Ailanthus altissima* (Mill.) Swingle, suggesting verifying the stability of the associated primary endosymbionts [20,21]. OSW-infested plants exhibit a notable reduction in photosynthesis and a gradual weakening caused by the sap-sucking behavior of nymphal stages. Furthermore, the nymphs produce considerable quantities of honeydew, promoting the development of sooty mold that can extensively envelop the leaves and fruits of the plant, posing an additional hazard to its overall health [22].

Due to the phytosanitary relevance of *A. spiniferus* in Italy and the whole EPPO region, and to monitor the spreading status of this invasive whitefly species, regular surveys on this insect in central and south Italy (namely in the regions Apulia, Campania, Lazio, Marche, and Sicily) were conducted. Given the efficacy of biological control of the OSW, primarily through the use of hymenopteran parasitoids, in several regions of the world [23–29], this monitoring activity carried out in Italy also included observations on the presence of natural enemies to evaluate the potential for natural biological control of OSW infestations and spread.

The OSW appears well controlled by natural enemies in its native areas [18,30]. Among the predators of *A. spiniferus*, species from the orders Diptera, Neuroptera, and Coleoptera (namely ladybugs) are worth mentioning [31], most of which exhibit polyphagous behavior and are active on other whiteflies, including *Aleurocanthus* species [18,30–32].

According to the literature, one native predator occurs in Italy from the family Coccinellidae, namely *Clitostetus arcuatus* (Rossi) [11]. Among the exotic ladybugs, two members of the Serangiini tribe also exist in Italy in association with the OSW: *Delphastus catalinae* (Horn) and *Serangium montazerii* Fürsch [11,33].

Several species of hymenopteran parasitoids associated with the OSW are known worldwide, the majority belonging to the family Aphelinidae (Chalcidoidea), in the genera *Encarsia* Förster and *Eretmocerus* Haldeman; the platigastrid *Amitus hesperidum* Silvestri has also been reported [31,34].

The genus *Eretmocerus* (Hymenoptera: Chalcidoidea: Aphelinidae) includes about 90 species [34], all known to be primary parasitoids of whiteflies with a cosmopolitan distribution [35]. Members of this parasitoid genus lay their eggs beneath their whitefly hosts. Upon hatching, the larvae of the parasitoid infiltrate the host's nymphs at different stages of their life cycle, developing as endoparasitoids [36]. The adults emerge from nymphs through an exit hole in the dorsum [37]. Historically, species such as *Eretmocerus serius* Silvestri and *Eretmocerus debachi* Rose and Rosen proved to be effective in international biological control measures aimed at the Citrus Blackfly and other whitefly pests, as *A. woglumi* and the bayberry whitefly, *Parabemisia myricae* (Kuwana) [23,38,39]. In addition, different *Eretmocerus* species were also introduced as natural enemies of the sweet potato whitefly, *Bemisia tabaci* (Gennadius), in Arizona, California, Florida, North Carolina, and Texas [40].

During regular surveys in Italy, the initial indication of an OSW parasitoid dates to 2018, marked by the discovery of a solitary holed puparium collected in Bari (Apulia) [41]. However, this finding needs more confirmation from subsequent discoveries. Starting from spring 2023, a significant and increasingly active parasitization over *A. spiniferus* has been detected almost contemporary in all five investigated regions, with collection from

multiple sites of numerous males and females of a parasitoid species belonging to the genus *Eretmocerus*.

Although their complete identification and molecular characterization are still ongoing, the morphological characteristics of all specimens collected in the five Italian regions mentioned above allow us to ascribe them to a single species belonging to the *Eretmocerus serius* group [32,42,43] and new to Europe.

This study presents initial data about the distribution and phenology of the *E.* sp. gr. *serius* in Italy, assessing its natural control efficacy against the OSW, considering the environmental features of the surveyed areas.

## 2. Materials and Methods

### *2.1. Field Collections*

Surveys have been carried out in 10 sites distributed in five regions of central-south Italy (Apulia, Campania, Lazio, Marche, and Sicily) overall from May 2023 to January 2024 (although with different starting of observations between different survey sites) on *Citrus limon* (L.) Burm. f., *Citrus sinensis* (L.) Osbeck, *Citrus* × *aurantium* L., and *Citrus clementina* Hort. ex. Tanaka (Table 1), trying to select plants on which chemical pesticides have not been applied.

**Table 1.** Localities, host plants, and frequencies of monitoring activities of *Aleurocanthus spiniferus* and *Eretmocerus* sp.gr. *serius* in central and south Italy carried out from May 2023 to January 2024.

| Localities | Coordinates | Host Plants | Starting Month | Frequency |
|---|---|---|---|---|
| | | **APULIA** | | |
| Bari (BA) | 41°06″ N; 16°52′ E | *Citrus sinensis* (L.) Osbeck, *Citrus limon* (L.) Burm. f. | July 2023 | Occasional |
| | | **CAMPANIA** | | |
| Casagiove (CE) | 41°04′ N; 14°19′ E | *Citrus limon* (L.) Burm. f. | May 2023 | Weekly |
| Piana di Monte Verna (CE) | 41°09′ N; 14°20′ E | *Citrus sinensis* (L.) Osbeck *Citrus limon* (L.) Burm. f. | July 2023 | Occasional |
| Portici (NA) | 40°48′ N; 14°21′ E | *Citrus limon* (L.) Burm. f. | May 2023 | Weekly |
| | | **LAZIO** | | |
| Terracina (LT) | 41°17′ N; 13°14′ E | *Citrus limon* (L.) Burm. f. | November 2023 | Occasional |
| Roma (RM) | 41°53′ N; 12°26′ E | *Citrus clementina* hort. ex Tanaka | January 2024 | Occasional |
| | | **MARCHE** | | |
| Grottammare (AP) | 42°59′ N; 13°52′ E | *Citrus* × *aurantium* L. *Citrus sinensis* (L.) Osbeck | August 2023 | Occasional |
| | | **SICILY** | | |
| Caltagirone (CT) | 37°14′ N; 14°30′ E | *Citrus* × *aurantium* L. | July 2023 | Monthly |
| Siracusa (SR) | 37°04′ N; 15°16′ E | *Citrus* × *aurantium* L. | July 2023 | Monthly |
| Palermo (PA) | 38°07′ N; 13°19′ E | *Citrus* × *aurantium* L. | July 2023 | Occasional |

Sampling was conducted weekly at two sites in Campania (Casagiove and Portici) and monthly at two sites in Sicily (Caltagirone and Siracusa). Additional data were collected through non-regular sampling at all other sites across the five investigated regions.

Each sampling in Apulia, Campania, Marche, and Sicily collected 30 fully developed leaves infested by OSW from newly developed shoots. In the Lazio region, the activity only consisted of direct field observations on infested host plants, to detect signs of parasitization over the blackfly.

Materials sampled in Campania were isolated in a storage box, hermetically sealed with a double envelope, and moved to the Department of Agriculture of UNINA or IPSP laboratories (both in Portici) to perform more in-depth analyses.

To avoid further spreading of the pest from demarcated areas, in Marche and Sicily, observations and counting on the sampled material were realized directly at each collection site using a portable field microscope (Andowl QY-X01 Digital Microscope); the host nymphs were then isolated in gelatin capsules.

### *2.2. Identification of the Parasitoid*

Specimens used for taxonomic studies were mounted on slides using balsam-phenol medium [44]. The original paper of Silvestri [32] and keys provided by Compere [45], Gerling [42], and Hayat [43] were used for the parasitoid identification.

### *2.3. Parasitization Rate*

Apparently healthy 4th-stage nymphs of the whitefly host were detached from the leaves as cohorts of 5–10 specimens. Specimens were isolated in natural gelatine capsules (13.59 mm × 5.57 mm), placed in sealed Petri dishes, and transferred to the laboratory. Samples were stored at 25 ± 2 °C, 65 ± 10% R.H., and 16:8 (L:D) photoperiod until the emergence of parasitoids.

Emerged parasitoids were killed in 70% ethanol and stored at −20 °C until observations and analyses. For each field collection, the sex ratio of parasitoids was calculated as in [46], while the active parasitization rate was calculated using the formula [47]:

$$\left[\frac{n^{\circ}\ Emerged\ parasitoids}{n^{\circ}\ Apparently\ healthy\ 4^{th}\ stage\ nymphs}\right] \times 100$$

Apparently healthy 4th-stage nymphs were chosen because host's black integument makes the identification active parasitization, signs of oviposition, or the presence of meconium difficult. In addition, the parasitoid emergence holes were not considered to prevent misattributing parasitization to other potentially active parasitoids in the same context.

### *2.4. Phenology*

Preliminary data on the phenology of *E.* sp. gr. *serius*, concerning its whitefly host, were collected only from the two sites (Casagiove and Portici) in Campania where samplings have been performed weekly. To this aim, for each collection, all 4th instar nymphs of the whitefly host, apparently healthy, were counted on each leaf and isolated, as mentioned in the previous paragraph, and the number of emerged parasitoids was registered.

### *2.5. Temperature Trend and Parasitization Percentage*

To investigate the effects of temperature on the parasitization percentage, the temperature trends of Casagiove and Portici were analyzed. These investigations were only focused on these two sites as they represent the locations where the samplings were carried out weekly and on which preliminary data about the phenology of the parasitoid were collected. Weather data concerning minimum, average, and maximum temperatures were collected from the Civil Protection Organisation of the Campania Region online database (https://centrofunzionale.regione.campania.it (accessed on 12 December 2023)). The data were selected from 1 October 2022 (seven months before the first sampling) to 28 October 2023 (the date of the last field collection in Campania). The data have been downloaded from the database of the stations of San Marco Evangelista (Province of Caserta) and Ercolano (Metropolitan City of Napoli). These weather stations were chosen for their proximity to the sampling sites, as Casagiove and Portici lack similar points of precision measurement. Some correlations about temperature trends before and during the field monitoring were processed and represented.

## 3. Results

### *3.1. Identification of the Parasitoid*

Morphological analysis on the slide-mounted specimens allowed us to identify the parasitoid as *Eretmocerus* belonging to the *serius* group (Figure 1). The species group, which this parasitoid can be ascribed, presently includes four species (Gerling, 1969; Hayat, 1998; Silvestri, 1927) and is characterized by the presence of a female's antennal club whole and highly developed, with subparallel margins and a rounded apex, as well as very small funicular segments, with the first one being ring-like and the second trapezoidal. The forewing is characterized by a short marginal fringe (Gerling, 1969).


**Figure 1.** (**A**) *A. spiniferus* 4th-stage nymph with circular exit hole; (**B**) *Eretmocerus* sp. gr. *serius* adult.

### *3.2. Parasitization Rate*

The results regarding the parasitization rate of OSW by *Eretmocerus* sp. gr. *serius* are reported below.

APULIA: In Bari (BA), a high whitefly population corresponded to a 4.07% parasitism, with a sex ratio value of 0.43 (Tables 2 and S1).

**Table 2.** Maximum parasitization level of *A. spiniferus* by *Eretmocerus* sp. gr. *serius* recorded in central and southern Italy.

| Localities | Max Parasitization Rate % (Date) |
|---|---|
| APULIA | |
| Bari (BA) | 4.07 (Jul 14) |
| CAMPANIA | |
| Casagiove (CE) | 51.43 (Jun 5) |
| Piana di Monte Verna (CE) | 63.16 (Oct 14) |
| Portici (NA) | 71.43 (Jun 5) |
| MARCHE | |
| Grottammare (AP) | 26.69 (Sep 20) |
| SICILY | |
| Caltagirone (CT) | 8.36 (Dec 19) |
| Siracusa (SR) | 9.66 (Nov 28) |
| Palermo (PA) | <1.00 (Sep 29) |

CAMPANIA: At the Casagiove site, the maximum rate of parasitization was 51.43%, recorded on 5 June 2023 (Figure 2, Table S2). A reduction in the parasitization rate was observed at the end of July 2023. Subsequently, there was a gradual increase until reaching a peak (25.33%) on August 5. In the period spanning from late August to September, the parasitization rate reached a minimum value of 2.60% and was followed by the last parasitization peak reached on 15 October scoring 20%.

The parasitization rate at the Portici site peaked on 5 June 2023, reaching a value of 71.43%. However, for the remainder of the year, only one other peak was recorded, with maximum values around 12% (Figure 2, Table S3).

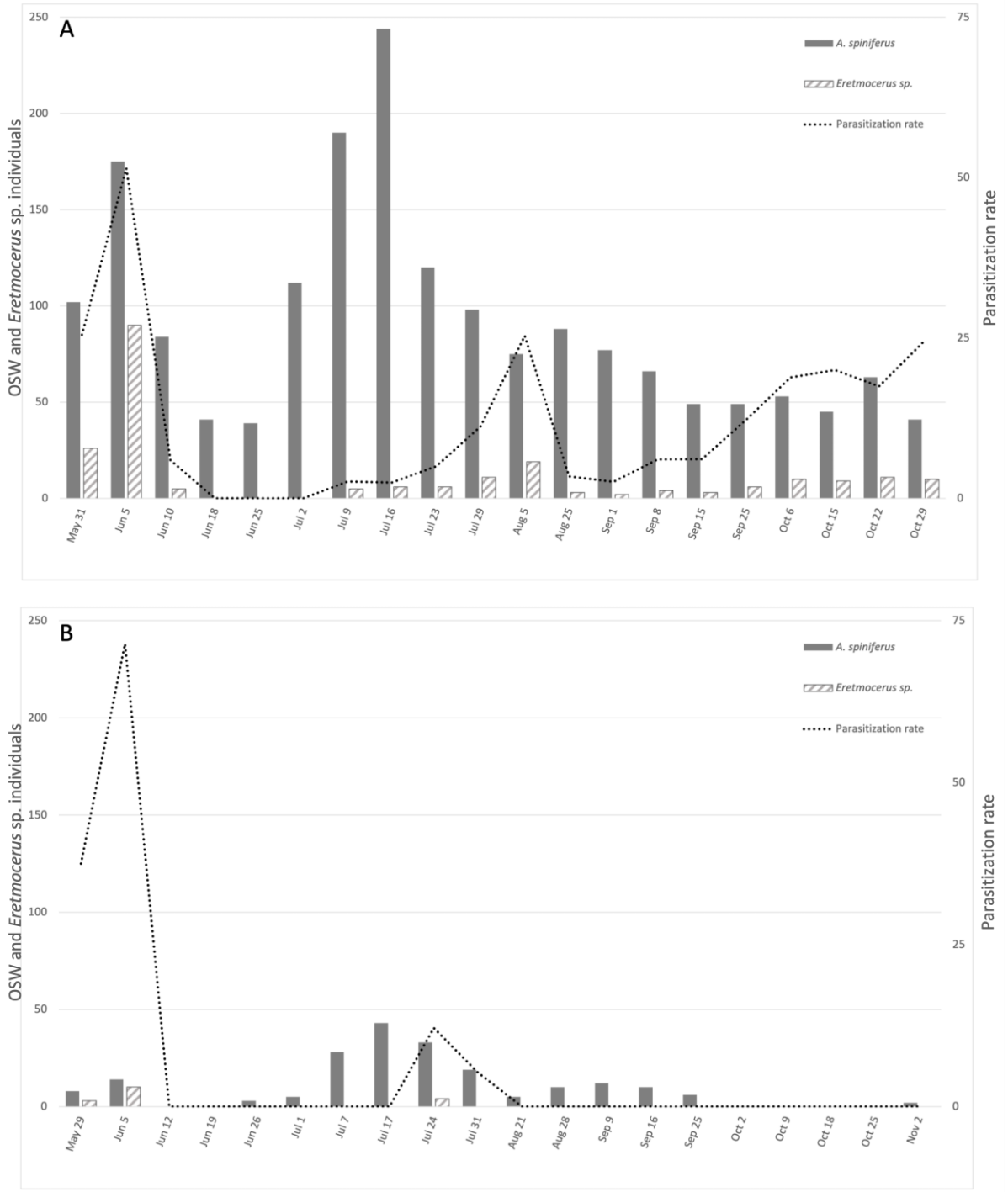


**Figure 2.** Population dynamics of *Eretmocerus* sp. gr. *serius* and *A. spiniferus* along with parasitization rate during 2023: (**A**) Casagiove site; (**B**) Portici site.

Despite the OSW infestation, no *Eretmocerus* sp. gr. *serius* parasitization was recorded from Piana di Monte Verna (CE) in mid-July 2023. A second sample returned a parasitization rate of 63.16% in mid-October. No males of the parasitoid emerged from the samples.

MARCHE: The highest host and parasitoid populations were in Grottammare (AP). The resulting parasitization rate was approximately constant, around 24.67% in August and 26.69% in September 2023, with sex ratio values of 0.49 and 0.66, respectively (Table S1).

SICILY: No parasitization by *Eretmocerus* sp.gr. *serius* or any other parasitoid has emerged in July and August in any of the sites investigated, distributed in the provinces of Caltanissetta, Catania, Messina, Palermo, and Siracusa, despite the very high levels of infestation by *A. spiniferus* (data under publication). The first discovery of the aphelinid in Sicily was made in late September 2023 at the Palermo (PA) site. However, it showed low levels of parasitization, scoring less than 1%. Immediately after, in October 2023, the presence of the parasitoid appeared almost contemporary for the first time in the sites of Caltagirone (CT) and Siracusa (SR), with parasitization rates raised up to almost 10% within two months.

LAZIO: In Terracina (LT) and Roma (RM), parasitization activity over *A. spiniferus* was recorded, respectively, in November 2023 and January 2024. Many fourth-stage nymphs showed the typical circular exit hole on the dorsum and the entry point of *Eretmocerus* spp.'s first larva on the ventral side, which testifies to the presence of parasitization by the aphelinid.

### *3.3. Phenology*

The population dynamics of OSW and *Eretmocerus* sp. gr. *serius* are shown in Figure 2. For both monitored sites, data show a peak of presence with a similar trend in the first ten days of June. In the following phase, from the end of June to the middle of July, the whitefly population in Casagiove (CE) reached a new maximum around mid-July.

The parasitoid sex ratio fluctuated during the observation interval, with a total mean value in Campania of 0.79. The males were almost always present, but with a greater incidence in mid-July, early September, and late October (Table S1).

### *3.4. Temperature Trends and Parasitization Percentage in Campanian Sites*

The temperatures showed a very similar trend during the data collection period and in the period prior to the first samplings (Figures S1–S3). The only difference in temperature trends between the two locations occurred in September when the average temperature in the Province of Caserta was slightly higher than that recorded in the Metropolitan City of Napoli (Figure S3).

The higher parasitization percentages observed in Portici and Casagiove coincided with the beginning of June, when the average temperature recorded by the weather stations in both locations was around 21 °C. The second peak of the presence of *Eretmocerus* sp. gr. *serius* occurred in the second half of July in both localities, with average temperatures ranging between 28 °C and 30 °C (Figures S1 and S2).

## 4. Discussion

The *Eretmocerus* sp. gr. *serius* finding represents the first report in Europe of a parasitoid associated with *A. spiniferus* since Porcelli's record of the blackfly in 2008. Little information exists in the literature on the biology of these parasitoids [38]. It was impossible to find or examine the type of material of *Eretmocerus serius* Silvestri, *Eretmocerus orientalis* Gerling, and *Eretmocerus silvestrii* Gerling. These types are no longer present in the Silvestri Museum of the University of Napoli because they were loaned in 1984 to Prof. Mike Rose of the Department of Entomology of Montana State University. To date, attempts to recover this type of material have been unsuccessful.

With the exception of a solitary holed puparium in Bari [41], *Eretmocerus* sp. gr. *serius* was never noticed in all surveys carried out in Italy during previous years on *A. spiniferus*.

However, it has appeared almost simultaneously between May 2023 and January 2024 in all the investigated central-southern regions of Italy.

Monitoring activities carried out on citrus plants showed an interesting parasitization activity in all the checked locations. The emergence curves of host-parasitoids seem to follow similar patterns in Portici and Casagiove.

From the end of June to mid-July, the whitefly population at the Casagiove site reached a new maximum around the beginning of August and, from the late summer and autumn, the parasitoid continued its activity. In the Portici site, the rarefaction of the host population and its natural enemy have made this trend less evident, although the presence of the host was less abundant than in previous years of observations on *Citrus* spp. [11]. Indeed, except for occasional presences, OSW has been absent in Portici since October 2023 (Figure 2). The drastic reduction of host populations is probably attributable to the activity impact of aphelinids, testified by numerous nymphs with the typical exit hole known for the species of the genus *Eretmocerus*, as well as to the predation activities by *S. montazerii*, *C. arcuatus*, and *D. catalinae*. Population trends of *Eretmocerus* sp. gr. *serius* indicate that even slight increases in its presence correspond to a clear decrease in the host population.

The parasitoids' actions could have significantly impacted the survival rates of the invasive species in the Campania region (Table 2). It should be noted that in this study, parasitism data only considered parasitoids that emerged from apparently healthy hosts.

In Apulia, the observation mid-July interval highlighted that the parasitization level was relatively low but consistent with the level observed in Casagiove during the same period (Table 2, Figure 2).

More whiteflies on citrus leaves in Grottammare (Marche) were observed compared to all other surveyed locations (Table S1). The higher population magnitude may be related to the recent pest arrival and outbreak. However, the parasitoid reached the host, giving the maximum number of individuals compared to all other localities, although the level of parasitization appeared to be slightly lower than the Campanian sites.

At the end of the summer, the parasitoid started to show its presence in western Sicily, namely in Palermo (PA), although in an almost negligible population. In just a couple of months, its parasitic activity has rapidly and strongly increased all over the region, and parasitization rates close to 10% have been registered almost simultaneously and already in November–December in eastern Sicily, in the two investigated sites of Caltagirone (CT) and Siracusa (SR). However, the population density of *A. spiniferus* has remained constantly abundant throughout the observation period in all the monitored localities.

The parasitization activity over *A. spiniferus* recorded in Rome in January 2024 is probably related to the same aphelinid species found in all the other studied Italian locations. The data on the parasitization activity by an *Eretmocerus* on the whitefly observed on November 2023 on lemon leaves in Terracina (LT) need validation as they are only based a photographic report.

The analysis of the charts representing temperature trends in correlation with the parasitization percentage suggests that temperature does not significantly influence the degree of parasitization of OSW by *Eretmocerus* sp. gr. *serius*. Interestingly, the maximum parasitization rate occurred around the same time in both locations, at the beginning of June, with an average temperature of around 21 °C; then, between the end of July and the beginning of August, there was a decrease in activity. For instance, some researchers have found that *E. serius* can effectively control *A. woglumi* during rainy seasons, although prolonged periods of drought can hinder the parasite's development and population [32,48,49].

Based on the collected data, the finding of *Eretmocerus* sp. gr. *serius* in Italy could represent a promising solution in the biological control of *A. spiniferus*, which, in recent years, has expanded its distribution throughout Italy, and especially in Europe, with a particular northward trend. The importance of *E.* sp. gr. *serius* lies primarily in its finding in Italian territories invaded by OSW, and therefore, it should not require numerous, in-depth surveys for its own introduction unlike other allochthonous parasitoids [50]. Following the regulations currently in place, the process of introducing a natural enemy is

lengthy and laborious, slowing down the chances of a prompt control and thus allowing the pest populations to further spread, cause damage, and shift to other new host-plants.

Morphological and molecular studies are underway to identify the species in a dedicated publication, and further studies and monitoring activities in the next few years will be relevant to better describe its geographical distribution and the impact of this natural enemy on OSW.

## 5. Conclusions

*Aleurocanthus spiniferus* is rapidly invading Europe and poses a significant threat to citrus crops, causing notable agricultural damage. The new finding of the parasitoid *Eretmocerus* sp. gr. *serius* can mark a crucial advancement in biological control strategies, showcasing biocontrol potential to manage invasive species successfully. *Eretmocerus* sp. gr. *serius*, belonging to an aphelinid genus that is well known for parasitizing whiteflies, could represent a natural solution to mitigate *A. spiniferus* infestations. This success underscores the intricate balance required for sustainable pest management in agricultural ecosystems, offering promise in safeguarding European citrus crops from the destructive impacts of the invasive OSW.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su16072970/s1, Table S1: Infestation and parasitization data in the localities of occasional collections along with sex ratio of the emerged *Eretmocerus* sp. gr. *serius*; na: not available data; Table S2: Infestation and parasitization data in Casagiove (CE) along with sex ratio of the emerged *Eretmocerus* sp. gr. *serius*; na: not available data. Table S3: Infestation and parasitization data in Portici (NA) along with sex ratio of the emerged *Eretmocerus* sp. gr. *serius*; na: not available data; Figure S1: Temperatures trend and percentage of parasitization in the province of Naples; Figure S2: Temperatures trend and percentage of parasitization in the province of Caserta; Figure S3: Average temperature in the provinces of Naples and Caserta.

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