

RAPID COMMUNICATION

Classical Biological Control of *Diaphorina citri* in Cyprus: High Parasitism, Population Suppression, and the First Record of the Hyperparasitoid, *Marietta leopardina*

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ABSTRACT

Asian citrus psyllid, *Diaphorina citri*, was first detected in Cyprus in 2023. This finding initiated an eradication campaign and a classical biological control program based on the introduced ectoparasitoid *Tamarixia radiata*. Recent integrative taxonomic work revealed the presence of a second primary parasitoid species, *Tamarixia citricola*, associated with *D. citri* nymphs in Cypriot citrus orchards. Here, we provide a post-release monitoring update on this biological control program based on field surveys conducted in late October 2025 at citrus sites selected because of previous records of *D. citri* or recent reports of psyllid activity from the Cypriot Plant Health Services. Overall, adult *D. citri* abundance was very low; adults were absent from nearly all orchards and were detected at clearly higher levels in only one site, where 60% of tap-sampled flush shoots were positive for adult presence. Nymphs showed a similar pattern, with only sparse, low-density colonies detected across the surveyed orchards. Importantly, the site with the highest *D. citri* density also showed the highest nymph parasitism (96.2%), consistent with strong parasitoid-associated suppression. Of the primary parasitoids that emerged, 96.9% were *T. radiata*, whereas 3.1% were *T. citricola*. For the first time in Cyprus, we also recorded the hyperparasitoid *Marietta leopardina*, which reached 26.7% hyperparasitism and showed a marked association with primary parasitoids developing in third-instar nymphs. These results support the important role of *T. radiata* in the current classical biological control program in Cyprus and underscore the need for continued monitoring to understand better emerging trophic interactions that may influence long-term outcomes.

1 | Introduction

Diaphorina citri Kuwayama (Hemiptera: Psyllidae), the Asian citrus psyllid, is the primary vector of *Candidatus Liberibacter*

asiaticus, the causal agent of huanglongbing (HLB), the most destructive disease of citrus worldwide (Bové 2006; Gottwald 2010; Pérez-Hedo, Hoddle, Alferez, Tena, et al. 2025). Although HLB has not been detected in the Mediterranean Basin, the recent

establishment and rapid spread of *D. citri* in Cyprus have raised substantial concern for the region's citrus industry (Pérez-Hedo, Hoddle, Alferez, Batista, et al. 2025). Following the detection of *D. citri* in Cyprus in 2023, an eradication program was initiated, but could not be fully implemented due to the widespread distribution of small citrus plantings, scattered orchards, and backyard trees across the island. Consequently, a classical biological control program targeting *D. citri* was launched in 2024 with the introduction of the nymphal ectoparasitoid *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae), sourced from California, U.S. (Melifronidou-Pantelidou et al. 2025).

During the first post-release monitoring surveys conducted in 2024, parasitised *D. citri* nymphs were detected in both *T. radiata* release and non-release orchards across Cyprus (Urbaneja et al. 2025). *Tamarixia radiata* was successfully recovered from sites where releases had been conducted, confirming its establishment in the field. However, parasitoids were also found in several orchards with no history of releases. These findings suggested that *T. radiata* had either dispersed rapidly from release sites or that additional unknown parasitoid species were attacking *D. citri* in Cyprus. An integrative approach using DNA barcoding (COI) and morphological analyses confirmed that parasitism in several non-release orchards was due to *Tamarixia citricola* Hansson & Guerrieri (Hymenoptera: Eulophidae), a previously unknown and subsequently newly described species (Urbaneja et al. 2025). This discovery revealed the coexistence of two parasitoids, introduced *T. radiata* and likely autochthonous *T. citricola*, attacking *D. citri* nymphs within the same agroecosystem. This finding has important implications for interpreting parasitism patterns and for designing future management strategies targeting *D. citri* in the Mediterranean region (Urbaneja et al. 2025).

In late October 2025, following the 2024 release and the early establishment phase of *T. radiata* previously documented in Cyprus (Urbaneja et al. 2025), a targeted post-release monitoring survey was conducted at citrus sites selected because of previous records of *D. citri* or recent reports of psyllid activity from the Cypriot Plant Health Services. This study aimed to assess the current detectable presence of *D. citri* in surveyed orchards, quantify nymph parasitism where infestations were present, determine the composition of the associated primary parasitoid complex, and document the occurrence of a hyperparasitoid within this system. The results provide an updated field assessment of this emerging biological control program in Cyprus.

2 | Materials and Methods

2.1 | Survey Rationale and Site Selection

This 2025 survey was designed as a targeted post-release monitoring assessment of citrus orchards with previous or recent records of *D. citri* in Cyprus, rather than as a formal release-versus-non-release comparison. The release history and early establishment phase of *T. radiata* in Cyprus during 2024, including the initial release sites and release numbers, were previously documented by Urbaneja et al. (2025). Briefly, the first releases of *T. radiata* were conducted in spring 2024 in Phassouri (site 13), Potamia (site 16), Frenaros (site 1), and Avgorou (site 2) (Figure 1). Additional releases were subsequently carried out in

November 28, 2024, at Peristonia (site 6) and the Green Zone (site 7), with 60 and 40 parasitoids released at the respective sites. These releases took place after the July 2024 field surveys reported by Urbaneja et al. (2025) and were intended to reinforce the establishment of the parasitoid in those areas. Because the main objective of the 2025 survey was to update the field status of *D. citri* and its associated parasitoids following this early establishment phase, orchards were selected based on previous infestation history or recent reports of *D. citri* activity.

2.2 | Study Sites

As part of the *D. citri*–*Tamarixia* monitoring program conducted from 28 October to 1 November 2025, a subset of the citrus orchards surveyed in 2024 was re-examined (Table 1 and Figure 1) (Urbaneja et al. 2025). Several orchards surveyed in 2024 were not resampled because preliminary inspections by the Cypriot Plant Health Services indicated no detectable *D. citri* populations at those sites during the preceding 3 months. Additionally, seven sites were included in the 2025 survey based on recent reports of *D. citri* presence from the Cypriot Plant Health Services or to expand monitoring in areas of interest. Together, these orchards represented historically infested or newly reported sites distributed across the main citrus-growing districts of Cyprus.

2.3 | Sampling Methods

Adult *D. citri* were surveyed using a standardised tap-sampling method focused on flush shoots, that is, the most recent vegetative growth and the plant structure most suitable for *D. citri* feeding, oviposition, and nymphal development (Monzó et al. 2015). To dislodge adult insects, shoots were gently struck over a black plastic tray, and the insects collected on the tray were immediately visually examined and identified in the field. For each orchard, 20 individual flush shoots were sampled by beat tray. Whenever tree availability allowed, one flush shoot was sampled per tree; when fewer trees were available, up to two flush shoots were sampled per tree, depending on tree size and flush availability. This sampling method allowed estimation of the proportion of beat-tray samples in which adult *D. citri* were detected, together with the mean number of individuals recorded per tap sample. Although highly mobile adult *D. citri* may occasionally escape detection immediately after disturbance, this method provides a rapid, standardised field estimate of adult presence (Monzó et al. 2015).

In each orchard, approximately 300–400 leaves were collected from young citrus flushes at phenological stages susceptible to *D. citri* infestation, with particular attention to flushes bearing advanced nymphal instars suitable for detecting parasitism. These leaves were taken from multiple trees distributed across each orchard, typically from 5 to 8 trees when availability allowed, according to flush availability, and transported to the laboratory in sealed plastic bags within coolers containing ice packs to preserve the viability of parasitised and unparasitised nymphs. Because *D. citri* nymphs develop exclusively on young flush, leaf-based assessments were restricted to tissue from the most recent vegetative growth, particularly fully expanded leaves where third- and later-instar nymphs are commonly

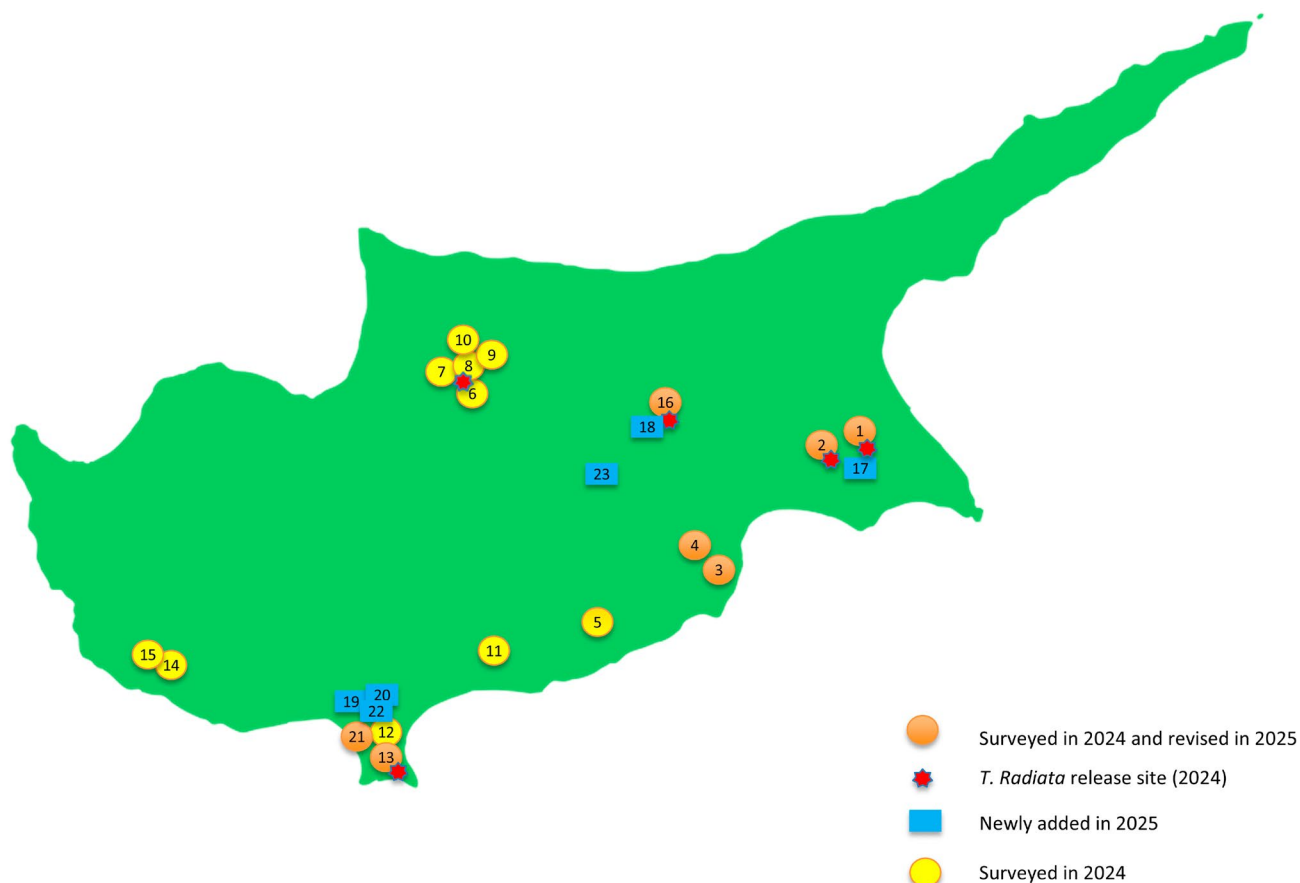


FIGURE 1 | Map of Cyprus showing the citrus orchards surveyed for *Diaphorina citri* and its parasitoids during the 2024 and 2025 monitoring surveys. Numbered points correspond to the sampling sites described in Table 1. Orange circles indicate sites surveyed in 2024 and directly resampled during the October 2025 survey; yellow circles indicate sites surveyed in 2024 but not directly resampled in October 2025; blue squares indicate sites newly added in 2025. Red stars indicate the sites where *Tamarixia radiata* was released in 2024 as part of the classical biological control program. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/jen.20136)]

found (Grafton-Cardwell et al. 2013; Pérez-Hedo et al. 2024). This approach allowed the detection of advanced nymphal stages suitable for assessing parasitism and provided a standardised operational measure for orchard-level comparisons within this monitoring survey.

Similarly, when ectohyperparasitoids were detected, the hyperparasitised *D. citri* nymphal instar was recorded directly at the time of leaf inspection, based on the presence of parasitised *Tamarixia* prepupae or pupae within the host nymph (Figure 2). This approach allowed the direct association of hyperparasitoid development with the specific host nymphal instar, without relying on mass rearing or post-emergence inference. Based on these observations, the percentage of infested leaves, the mean number of *D. citri* nymphs per leaf, parasitism rate, hyperparasitism rate, and the distribution of parasitism and hyperparasitism across *D. citri* nymphal instars were calculated for each orchard.

2.4 | Parasitoid Emergence and Preservation

Leaves bearing parasitised *D. citri* nymphs were individually or collectively placed in ventilated emergence containers and maintained under controlled laboratory conditions until adult

parasitoids emerged. The remaining leaves not used for direct stereomicroscope assessments were placed in 60×60×60 cm methacrylate cages, with one side fitted with an entomological sleeve that allowed emerging adults to be aspirated. Emergence cages were maintained under controlled laboratory conditions (~27°C, 55% RH, and L:D 11:13h) and checked periodically. Emerged parasitoids were collected using an aspirator and initially sorted according to external morphology as primary parasitoids or potential hyperparasitoids. Adult *Tamarixia* spp. specimens were either preserved in 70% ethanol for classical taxonomic analysis or in 96% ethanol for molecular analyses. Emerged hyperparasitoids were similarly preserved in ethanol and subsequently subjected to morphological and molecular identification.

2.5 | Morphological Characterisation

In total, 158 adult parasitoid specimens were examined morphologically. For morphological characterisation, adult parasitoids were first examined under a stereomicroscope and sorted according to external morphology. Representative specimens were prepared as card-mounted material by dehydrating them in a xylene: absolute ethanol solution (1:1) for 24 h, followed by transfer to amyl acetate for a further 24 h. Specimens were then

TABLE 1 | Sampling sites surveyed for *Diaphorina citri* and parasitoid presence in Cyprus during the October 2025 monitoring program.

Site	Municipality	District	Coordinates	Variety	% ACP-infested flushes July 2024	Tap sampling		Leaf sampling			
						% with ACP adults (#/tap)	% ACP Infested leaves (n)	# ACP/leaf	% Parasitism	% Hyperparasitised	%
1	Frenaros	Famagusta	35°02'31.1" N 33°52'51.3" E	Valencia	100.0 ± 0.0	0.0 ± 0.0	5.0 ± 2.4 (80)	0.10 ± 0.03	87.5 ± 3.7	0.0 ± 0.0	0.0 ± 0.0
2	Avgorou	Famagusta	35°02'02.5" N 33°49'45.3" E	Lafa (Valencia type), clementines	100.0 ± 0.0	0.0 ± 0.0	3.1 ± 2.1 (65)	0.03 ± 0.02	100 ± 0.0	50 ± 5.0	50 ± 5.0
3	Meneou	Larnaca	34°51'32.6" N 33°35'47.8" E	Valencia, lemon	10.0 ± 4.7	0.0 ± 0.0	0.0 ± 0.0 (100)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
4	Meneou (2)	Larnaca	34°51'34.5" N 33°35'49.6" E	Different varieties	10.0 ± 6.7	0.0 ± 0.0	0.0 ± 0.0 (100)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
5	Agios Theodoros	Larnaca	34°45'54.2" N 33°24'02.2" E	Navel, Clementines	100.0 ± 0.0	—	—	—	—	—	—
6	Peristerona	Nicosia	35°08'00.7" N 33°04'52.3" E	Different varieties	25.0 ± 9.7	—	—	—	—	—	—
7	Green zone	Nicosia	35°09'47.0" N 33°03'02.3" E	Sour orange	100.0 ± 0.0	—	—	—	—	—	—
8	Peristerona	Nicosia	35°08'23.5" N 33°05'26.6" E	Tango	15.0 ± 5.6	—	—	—	—	—	—
9	Avlona	Nicosia	35°09'07.0" N 33°06'45.7" E	Valencia, Lemon	5.0 ± 4.9	—	—	—	—	—	—
10	Avlona 2	Nicosia	35°09'19.4" N 33°06'27.9" E	Valencia, Lemon	10.0 ± 4.7	—	—	—	—	—	—
11	Pyrgos	Limassol	34°44'49.7" N 33°11'08.3" E	Clementines, Lemon	80.0 ± 8.9	—	—	—	—	—	—
12	Monagroulli	Limassol	34°38'28.9" N 32°57'00.7" E	Kaffir lime	10.0 ± 4.7	—	—	—	—	—	—
13	Phassouri	Limassol	34°38'09.1" N 32°56'54.4" E	Clementine	80.0 ± 8.9	0.0 ± 0.0	0.0 ± 0.0 (100)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

(Continues)

TABLE 1 | (Continued)

Site	Municipality	District	Coordinates	Variety	% ACP- infested flushes July 2024	Tap sampling	Leaf sampling			
						% with ACP adults (#/tap)	% ACP infested leaves (n)	# ACP/ leaf	% Parasitism	% Hyperparasitised
14	Anarita	Paphos	34°43'37.4" N 32°31'28.5" E	Orange, Clementines, Lemon	15.0 ± 5.6	—	—	—	—	—
15	Acheleia	Paphos	34°44'31.4" N 32°28'52.9" E	Lemon	20.0 ± 6.3	—	—	—	—	—
16	Potamia	Nicosia	35°03'54.6" N 33°27'27.9" E	Washington Navel	10.0 ± 4.7	0.0 ± 0.0	0.0 ± 0.0 (100)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
17	Liopetri	Famagusta	34°59'44.1" N 33°52'09.5" E	Lime	—	60.0 ± 11.0 (0.9 ± 0.2)	73.0 ± 5.2 (74)	2.1 ± 0.6	96.2 ± 2.2	26.7 ± 4.5
18	Potamia	Nicosia	35°03'56.2" N 33°27'27.6" E	Navel	—	25.0 ± 9.7 (0.2 ± 0.1)	6.0 ± 2.4 (100)	0.2 ± 0.03	38.9 ± 4.9	0.0 ± 0.0
19	Episkopi	Limassol	34°40'01.5" N 32°54'30.5" E	Sour orange	—	5.0 ± 4.9 (0.05 ± 0.05)	2.0 ± 2.0 (50)	0.02 ± 0.02	100.0 ± 0.0	0.0 ± 0.0
20	Episkopi	Limassol	34°40'01.5" N 32°54'38.3" E	Lemon	—	5.0 ± 4.9 (0.05 ± 0.05)	0.0 ± 0.0 (100)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
21	Phassouri	Limassol	34°37'48.3" N 32°56'55.3" E	Grapefruit	—	0.0 ± 0.0	0.0 ± 0.0 (100)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22	Asomatos	Limassol	34°38'24.8" N 32°56'49.3" E	Ortanique	—	0.0 ± 0.0	0.0 ± 0.0 (100)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
23	Pergamos	Larnaca	34°59'37.7" N 33°24'03.7" E	Mixed unidentified varieties	—	5.0 ± 4.9 (0.05 ± 0.05)	0.0 ± 0.0 (100)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Note: Sites 1–16 correspond to orchards previously surveyed in 2024, whereas sites 17–23 were included in the 2025 survey based on recent reports of *D. citri* activity from the Cypriot Plant Health Services or to expand monitoring in areas of interest. The table provides the location of each orchard, including municipality, district, and GPS coordinates; citrus varieties present; percentage of ACP-infested flushes recorded in July 2024; tap-sampling results; and leaf-sampling data. Tap-sampling data include the percentage of flush shoots with *D. citri* adults and, in parentheses, the mean number of adults per tap. Leaf-sampling data include the percentage of leaves infested by *D. citri* nymphs, with the number of leaves examined indicated in parentheses, the mean number of nymphs per leaf, percentage of parasitised nymphs, and percentage of hyperparasitism. Values are expressed as mean ± SE. Dashes for sites 1–16 indicate orchards that were not resampled in October 2025 because the Cypriot Plant Health Services had previously inspected them and had not detected *D. citri* during the preceding 3 months. Although these records could be interpreted as zero values, they are reported as dashes because no direct sampling was conducted during the October 2025 survey. In the remaining cases, dashes indicate that the corresponding data were not available.

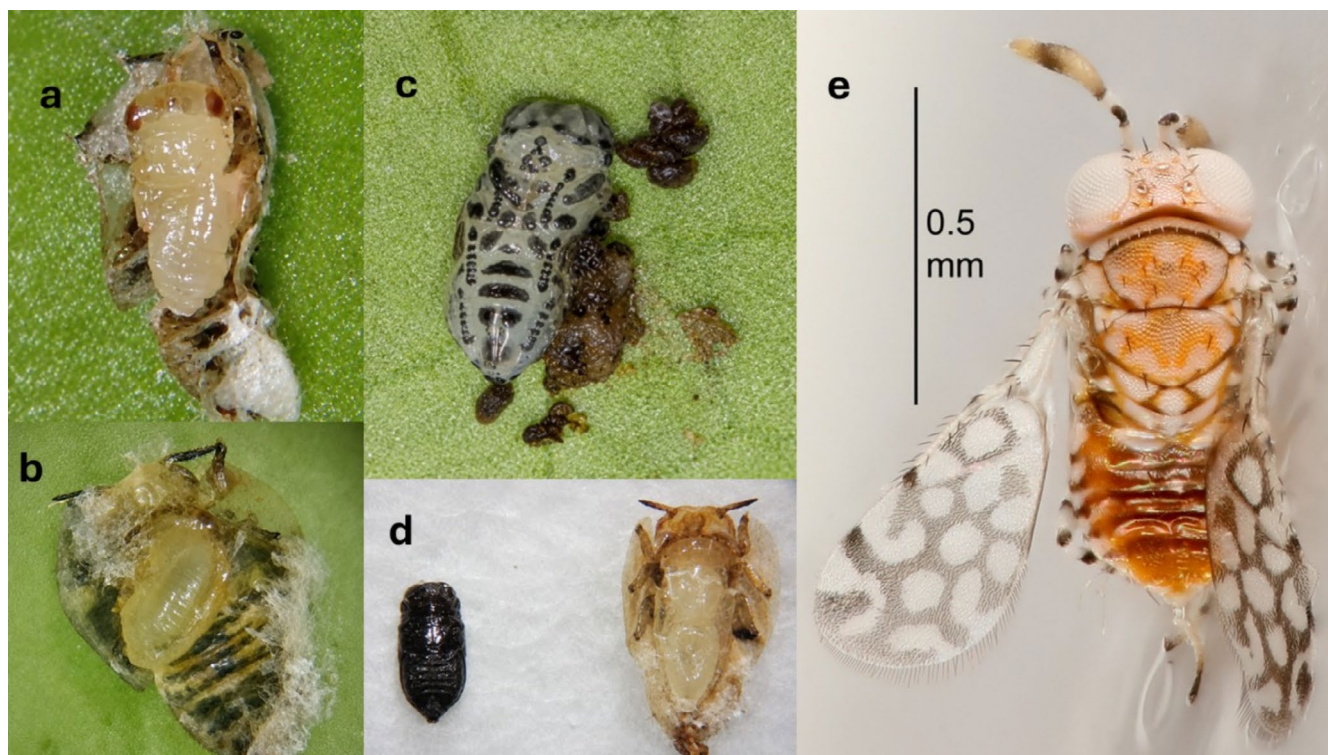


FIGURE 2 | Developmental stages and adult morphology of the hyperparasitoid *Marietta leopardina* associated with *Tamarixia* spp. parasitizing *Diaphorina citri* in Cyprus. (a, b) Larvae of *M. leopardina* feeding externally on *Tamarixia* spp. prepupae, (c) Newly formed pupa of *M. leopardina* showing the characteristic pale patterning typical of the species, (d) Mature, dark pupal stage of *M. leopardina* (left) compared with a pupa of *Tamarixia radiata* (right) for reference, (e) Adult female *M. leopardina*, highlighting the distinctive coloration and wing patterning of the species. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

placed in fresh amyl acetate and, after evaporation, mounted on card points using Arabic gum.

From the series of card-mounted material, three representative specimens were selected for slide mounting to allow detailed examination of diagnostic morphological characters. Slide preparations followed the protocol described by Noyes (1982). Briefly, specimens were macerated in KOH on a hot plate at 100°C for approximately 3 min, transferred to acetic acid for 5 min, and dehydrated through an ethanol series of 70%, 80%, 95%, and 100% ethanol, with each step lasting 5 min. Once in absolute ethanol, a drop of clove oil was added, and after evaporation of the alcohol, the specimens were dissected in clove oil and slide-mounted in Canada balsam. Dissection and slide mounting were performed to examine diagnostic characters required for reliable species-level identification.

Parasitoid identifications were performed using available taxonomic keys and reference resources (Anneck and Insley 1972; Hayat 1997; Myartseva et al. 2001; UCD Community 2023). Card-mounted and slide-mounted specimens were also compared with authoritatively identified reference material deposited at the Natural History Museum, London, UK.

2.6 | Molecular Characterisation

For molecular characterisation, mitochondrial genomic DNA was extracted from individual parasitoids using a modified

salting-out protocol optimised for small arthropods, as described by Monzó et al. (2011). Molecular analyses were conducted on a subset of emerged adults obtained from parasitised *D. citri* nymphs collected at Liopetri (site 17), the orchard with the highest *D. citri* density and parasitism levels during the 2025 survey. Individuals were initially assigned to morphogroups based on external morphology and labelled as putative *Tamarixia* sp. or putative *Marietta* sp. In total, COI sequences were analysed from 33 putative *Tamarixia* sp. individuals and five specimens visually assigned to *Marietta* sp.

Fragments of the mitochondrial cytochrome *c* oxidase subunit I gene (*COI*, also referred to as *COX1*) were amplified by polymerase chain reaction (PCR). For *Tamarixia* spp., a fragment from the 3' region of *COI* was amplified using the primers C1-J-2183, also known as JERRY (5'-CAACATTATTTTGATT TTTGG-3'), and TL2-N-3014, also known as PAT (5'-TCCAAT GCACTAATCTGCCATATTA-3') (Simon et al. 1994), generating partial sequences of approximately 800 bp. For the putative hyperparasitoid, two overlapping *COI* fragments corresponding to the 5' and 3' regions were amplified using the primer pairs LCO2/HCOoutout2 and JERRY/PAT, respectively. These fragments were Sanger-sequenced and assembled into a single consensus *COI* sequence of 1489 bp (GenBank accession PX965898) (Supporting Information).

PCR reactions were performed using Phusion Hot-Start Taq DNA Polymerase and contained 2 µL of mitochondrial DNA, 1× Phusion buffer including 1.5 mM MgCl₂, 0.2 mM of each dNTP,

and 0.2 μ M of each primer. The thermal cycling protocol consisted of an initial denaturation at 94°C for 2 min, followed by 10 cycles of 94°C for 15 s, 50°C for 15 s, and 68°C for 15 s, and then 40 additional cycles of 94°C for 15 s, 55°C for 15 s, and 68°C for 15 s. PCR products were visualised on 1.5% agarose gels to confirm amplification of fragments of the expected size and were subsequently purified and Sanger-sequenced using the same primers used for amplification.

Obtained sequences were inspected, trimmed to remove primer sequences, assembled into consensus sequences, and compared with reliable reference sequences available in public databases to support species identification. Phylogenetic relationships were inferred based on the *COI* barcode region using the Maximum Likelihood method under the General Time Reversible (GTR) model of nucleotide substitution. The robustness of the inferred topology was assessed by bootstrap analysis with 1000 replicates. Sequence alignment and phylogenetic analyses were conducted in MEGA version 12.

2.7 | Statistical Analyses

Given the low incidence of *D. citri* in most surveyed orchards, the 2025 monitoring survey was analysed primarily using descriptive statistics. For each orchard, percentages and mean values are presented with their corresponding standard errors where appropriate. Because only a few sites had detectable *D. citri* nymphal infestations, formal inferential comparisons among districts or orchard categories were not considered biologically meaningful for most variables. Therefore, the survey is presented as a targeted field assessment of the detectable distribution of *D. citri*, parasitism, and hyperparasitism across the sampled orchards.

3 | Results

During the October 2025 monitoring survey, *D. citri* incidence was generally low across the surveyed citrus orchards in Cyprus, although marked differences were observed among sites (Table 1; Figure 1). In July 2024, ACP-infested flushes were recorded in all 16 surveyed orchards, indicating *D. citri* presence at 100% of sites, with a mean of $43.1\% \pm 10.2\%$ infested flushes per orchard. In contrast, during the October 2025 survey, adult *D. citri* were detected by tap sampling in only 5 of the 13 directly sampled orchards, corresponding to 38.5% of sampled sites, with a mean of $7.7\% \pm 4.9\%$ positive tap samples per orchard when zero values were included. Adult detections were generally very low, with three of the five positive orchards showing only $5.0\% \pm 4.9\%$ of positive tap samples. Liopetri was the only site showing comparatively higher adult activity, with $60.0\% \pm 11.0\%$ of tap samples positive for adults. Although sampling approaches differed between years, these descriptive data indicate a marked reduction in the frequency of sites with detectable *D. citri* activity during the 2025 monitoring survey. Among the sites previously surveyed in 2024, only a subset was directly resampled in October 2025. Sites 5–12 and 14–15 were not directly sampled during the October 2025 survey because previous inspections by the Cypriot Plant Health Services had not detected *D. citri* in these orchards

during the preceding 3 months. Therefore, these records are reported as dashes in Table 1 rather than as zero values.

Among the orchards previously surveyed in 2024 and directly resampled in October 2025, adult *D. citri* were not detected by tap sampling. Nymphal populations were also absent or extremely scarce, being detected only at sites 1 and 2. Despite the low number of nymphs, parasitism was high at both sites, reaching 87.5% at site 1 and 100% at site 2. Hyperparasitism was not detected at site 1, whereas it was recorded at site 2, although this estimate was based on very few nymphs.

Among the sites newly included in the 2025 monitoring survey, *D. citri* occurrence was generally low, except at Liopetri (site 17). Adult detection by tap sampling was low at Potamia (site 18), Episkopi (sites 19 and 20), and Pergamos (site 23), and no adults were detected at Phassouri (site 21) or Asomatos (site 22). Nymphal populations were absent or scarce in most of these sites, and parasitism was detected where nymphs were available for assessment. Liopetri was the only orchard showing comparatively higher *D. citri* abundance and is therefore described separately below.

Liopetri (site 17) was the only orchard where *D. citri* occurred at comparatively higher abundance. At this site, $60.0\% \pm 11.0\%$ of tap samples were positive for *D. citri* adults, with 0.9 ± 0.2 adults per tap. Leaf infestation was also the highest recorded during the survey, with $73.0\% \pm 5.2\%$ of inspected leaves infested and a mean density of 2.1 ± 0.6 nymphs per leaf. Despite the higher pest abundance, parasitism was very high at $96.2\% \pm 2.2\%$. Hyperparasitism was also detected at this site, with a frequency of $26.7\% \pm 4.5\%$.

Laboratory emergence and taxonomic and molecular identification of parasitoids from the Liopetri samples confirmed the presence of both *T. radiata* and *T. citricola*. A total of 128 *Tamarixia* spp. adults emerged from field-collected samples. Of these, 124 were identified as *T. radiata* (92 females and 32 males), whereas four individuals were identified as *T. citricola* (three females and one male). Overall, *T. radiata* accounted for 96.9% of emerged *Tamarixia* adults, whereas *T. citricola* accounted for 3.1%. These results indicate a strong predominance of *T. radiata*, the introduced parasitoid species, within the sampled parasitoid community. Both species showed a female-biased sex ratio.

Analysis of parasitised *D. citri* nymphs from Liopetri showed that most parasitised hosts were fourth-instar nymphs, which accounted for 85% of all parasitised individuals. Third- and fifth-instar nymphs represented 7% and 8%, respectively. Thus, under the conditions observed at this site, parasitism by *Tamarixia* spp. was mainly associated with fourth-instar nymphs.

Within the highly parasitised *D. citri* population at Liopetri, hyperparasitism of *Tamarixia* spp. developing on parasitised *D. citri* nymphs was detected for the first time in Cyprus. Hyperparasitism was associated with a single ectohyperparasitoid species. The specimens analysed were collected from the Liopetri samples and emerged from *Tamarixia* sp. developing on *D. citri*. Among the sexed individuals, six were females, and two were males. All analysed individuals yielded

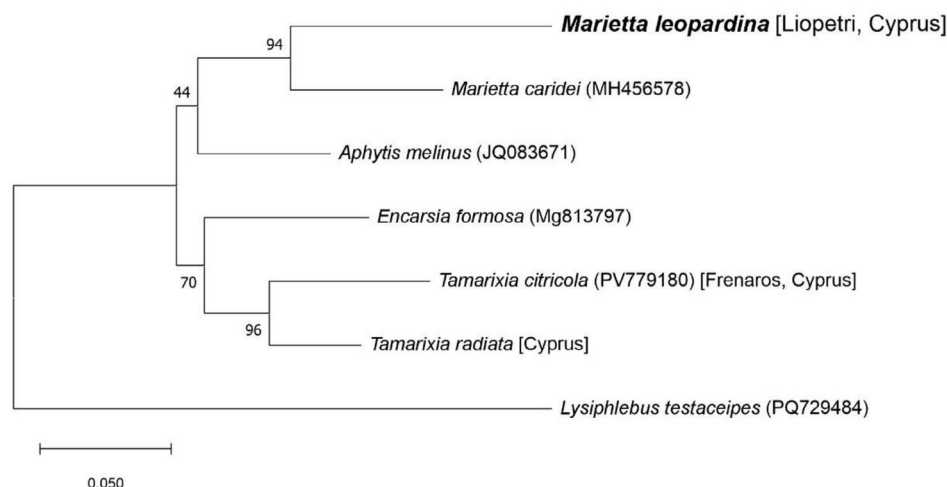


FIGURE 3 | Phylogenetic relationships inferred from COI 5' (barcode region) sequences of *Marietta leopardina* and related Chalcidoidea. The analysis includes *Marietta caridei* Blanchard as the focal genus, closely related Aphelinidae (*Encarsia formosa* Gahan and *Aphytis melinus* DeBach) to provide phylogenetic context, and *Tamarixia radiata* (Waterston) and *Tamarixia citricola* Hansson & Guerrieri (Eulophidae) to illustrate their separation from the hyperparasitoid lineage. The *COX1* sequence of the braconid *Lysiphlebus testaceipes* (Cresson) was included as an outgroup.

identical COI sequences, indicating a single mitochondrial haplotype in the sampled population. Two overlapping COI fragments, approximately 809 and 826 bp, were successfully amplified and assembled into a single 1489 bp consensus sequence. Database comparisons did not provide an unambiguous species-level match. However, based on diagnostic morphological characters, the specimens were identified as *Marietta leopardina* Motschulsky (Hymenoptera: Aphelinidae) (Figure 2). Phylogenetic analysis of the COI 5' region placed the Cypriot specimens within the genus *Marietta*, clustering with *M. caridei* and clearly separated from other Aphelinidae and from *Tamarixia* spp. (Figure 3).

Hyperparasitism showed a different distribution among host stages compared with primary parasitism. Although fourth-instar nymphs were the main stage parasitised by primary parasitoids, hyperparasitism was highest in third-instar nymphs, reaching 90%, lower in fourth-instar nymphs, reaching 24%, and was not detected in fifth-instar nymphs. The identity of the primary parasitoid host attacked by *M. leopardina*, either *T. radiata*, *T. citricola*, or both species, could not be determined from the available data. To our knowledge, this represents the first record of *M. leopardina* associated with the *D. citri*–*Tamarixia* system in Cyprus.

4 | Discussion

The results of the October 2025 monitoring survey provide field evidence consistent with a marked suppression of *D. citri* populations in Cyprus following the implementation of the classical biological control program with *T. radiata*. Although comparisons between 2024 and 2025 should be interpreted cautiously because sampling approaches differed between years, ACP-infested flushes were recorded in all 16 orchards surveyed in July 2024 (Urbaneja et al. 2025). In contrast, adult *D. citri* were detected by tap sampling in only 38.5% of the directly sampled orchards in October 2025. Moreover, *D. citri* populations were extremely scarce across most monitored sites, and even in Liopetri, the orchard where the pest was comparatively most abundant, parasitism exceeded 96%.

These results suggest that *Tamarixia* spp., and particularly the introduced *T. radiata*, are contributing substantially to the reduction of *D. citri* populations in Cypriot citrus orchards.

Because this survey was designed as a targeted post-release monitoring assessment rather than as a replicated comparative experiment, the results should be interpreted as a descriptive snapshot of the *D. citri*–*Tamarixia* system in late October 2025. The low incidence of *D. citri* and the concentration of nymphal populations in only a few sites limited the possibility of robust inferential comparisons among districts, release histories, or orchard categories. In addition, differences in sampling approaches between the 2024 and 2025 surveys mean that interannual comparisons should be interpreted cautiously. Nevertheless, the combination of low pest abundance across most monitored sites, high parasitism in areas where nymphs were present, and the recovery of *T. radiata* provides useful applied evidence for evaluating the early trajectory of the classical biological control program in Cyprus.

Similar successful outcomes following *T. radiata* establishment have been reported for *D. citri* infesting citrus on other islands, including Guadeloupe, Reunion Island, and Puerto Rico (Aubert and Quilici 1984; Étienne et al. 2001; Pluke et al. 2008). The recovery of both *T. radiata* and *T. citricola* from the Liopetri samples confirms the coexistence of two primary parasitoid species within the *D. citri*–citrus system in Cyprus. However, emergence data clearly indicated the predominance of *T. radiata*, which accounted for 96.9% of the emerged *Tamarixia* adults, whereas *T. citricola* accounted for only 3.1%. This pattern suggests that the introduced parasitoid has become the dominant primary parasitoid in the sampled population, while *T. citricola* persists at low relative abundance. A comparable pattern has been observed in the classical biological control program against *D. citri* in urban California, where post-release evaluations showed that *T. radiata* became the predominant and more successful parasitoid. In contrast, *Diaphorencyrtus aligarhensis* (Shafee, Alam & Agarwal) (Hymenoptera: Encyrtidae) established poorly or contributed little to overall parasitism (Milosavljević

et al. 2022). Although the Cypriot system involves a different co-occurring primary parasitoid, *T. citricola*, the apparent predominance of *T. radiata* in the present survey shows an interesting parallel with the California program. However, because the Cypriot data are still based on limited post-release monitoring, this pattern should be interpreted cautiously. The low recovery of *T. citricola* in October 2025 should therefore not be taken as evidence of limited ecological relevance, but rather as an early observation that requires further temporal monitoring. As observed in other *D. citri* classical biological control programs, the long-term contribution of co-occurring primary parasitoids may change over time and will likely depend on local ecological conditions, host availability, inter-specific interactions, seasonal dynamics, and the potential influence of hyperparasitoids.

The recovery of *Tamarixia* adults under conditions of generally low *D. citri* incidence also suggests that these parasitoids may persist and continue host-seeking when host availability is reduced, an important trait for the long-term stability of classical biological control programs (Hoddle et al. 2022; Milosavljević et al. 2021).

Within this apparently effective biological control system, the detection of the hyperparasitoid *M. leopardina* (syn. *M. javensis*) introduces a new trophic layer with potential implications for program durability. *Marietta leopardina* is an obligate hyperparasitoid that develops on primary parasitoids, including *Tamarixia* spp. and *Diaphorencyrtus* spp., attacking late larval or pupal stages within parasitised psyllid nymphs (Hoddle et al. 2013; Pérez-Rodríguez et al. 2019). Previous studies have shown that *M. leopardina* does not develop on unparasitised *D. citri* and that a substantial proportion of attacked *Tamarixia* larvae may die without yielding either a primary parasitoid or a hyperparasitoid, probably due to host feeding or aborted development (Hoddle et al. 2013). Reported hyperparasitism rates by *M. leopardina* on *T. radiata* in the native range of *D. citri* are typically low, usually below 10%, and total hyperparasitism remains below 10%–15% when other hyperparasitoid species are considered (Chen and Stansly 2014; Hoddle et al. 2014; Hoddle and Pandey 2014). In this context, the 26.7% hyperparasitism recorded at Liopetri is noteworthy and should be carefully monitored, particularly because it occurred at the site with the highest *Tamarixia* activity. However, this level of hyperparasitism was observed alongside exceptionally high primary parasitism, indicating that *Tamarixia* populations were abundant and may consequently have been more exposed to *M. leopardina*.

Further monitoring of *M. leopardina* and associated hyperparasitism rates will be required to determine whether the relatively high hyperparasitism observed in Liopetri represents a temporary local phenomenon or whether sustained hyperparasitism could eventually affect the long-term balance between primary parasitoids and hyperparasitoids. Such changes could, over time, reduce the effectiveness of biological control and allow *D. citri* populations to increase. The recent recovery of the hyperparasitoid *Cheiloneurus swezeyi* (Ashmead) (Hymenoptera: Encyrtidae) in the well-established *Metcalfa pruinosa*–*Neodryinus typhlocibae* system in northern Italy illustrates the importance of continuous monitoring of newly established trophic associations (Mazzon et al. 2025). In the present study, it was not possible to determine

whether *M. leopardina* attacked *T. radiata*, *T. citricola*, or both species. In addition, field collections and laboratory rearings indicated that hyperparasitism was highest in parasitoids developing within third-instar *D. citri* nymphs. In contrast, primary parasitism was mainly associated with fourth-instar nymphs. This suggests that the effects of hyperparasitism may be unevenly distributed among mixed-age *Tamarixia* cohorts and possibly between primary parasitoid species. These complex interactions between *D. citri*, two primary parasitoids, and *M. leopardina* may influence the long-term regulatory dynamics of the system and warrant further investigation (Hoddle et al. 2013; Bistline-East and Hoddle 2016; Vankosky and Hoddle 2019).

From a broader Mediterranean perspective, the findings presented here have important practical implications. Cyprus, together with Israel, currently represents one of the few known foci of *D. citri* establishment in the region (Pérez-Hedo, Hoddle, Alferez, Batista, et al. 2025), making these systems critical early indicators of risk for other citrus-producing countries in the Mediterranean Basin. The high parasitism rates documented in Cyprus indicate that classical biological control, when implemented early, can substantially reduce *D. citri* populations even in heterogeneous agricultural landscapes. At the same time, the first record of *M. leopardina* associated with the *D. citri*–*Tamarixia* system in Cyprus shows that resident or regionally widespread hyperparasitoids may rapidly become associated with introduced or locally established parasitoids in newly formed *D. citri*–*Tamarixia* systems. If *D. citri* spreads to neighbouring countries, understanding the *D. citri*–*Tamarixia*–*Marietta* dynamics observed in Cyprus will be essential for designing realistic biological control strategies and for anticipating both the suppressive potential of primary parasitoids and the possible constraints imposed by hyperparasitoids across the Mediterranean Basin.

Author Contributions

Alberto Urbaneja: conceptualisation, funding acquisition, investigation supervision, writing – original draft, manuscript revision. **Meritxell Perez-Hedo:** conceptualisation, methodology, formal analysis, investigation. **Nicos Seraphides:** conceptualisation, investigation, resources, manuscript revision. **Anthemis Melifronidou-Pantelidou:** conceptualisation, funding acquisition, resources, supervision, manuscript revision. **Stalo Giallouridou:** investigation. **Marios Georgiades:** investigation. **Menelaos C. Stavrinides:** investigation, manuscript revision. **Chrysos Kaponas:** investigation, manuscript revision. **Omar Ruiz-Rivero:** formal analysis, investigation. **Jose Catalan:** formal analysis, methodology, investigation. **David J. W. Morgan:** investigation, resources, manuscript revision. **Christer Hansson:** investigation, methodology, manuscript revision. **Mark S. Hoddle:** conceptualisation, investigation, manuscript revision. **Alejandro Tena:** conceptualisation, methodology, manuscript revision. **Emilio Guerrieri:** investigation, methodology, manuscript revision.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data generated or analysed during this study are included in this published article. The DNA sequences generated in this study have been deposited in a public repository and are available in GenBank under accession number PX965898.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** jen70136-sup-0001-supinfo.docx.