



The hyperparasitoid *Marietta picta* mediates the coexistence of primary parasitoids of goji berry psyllid

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With 2 figures

Abstract: Hyperparasitoids could disrupt the biological control system established by primary parasitoids. In Ningxia, China, accidentally introduced *Marietta picta* Andre was found since 2016 to hyperparasitize the primary parasitoid *Tamarixia lyciuni* Yang, a native biocontrol agent against the goji berry psyllid *Paratrioza sinica* Yang et Li. Self-introduced *Psyllaephagus arenarius* Trjapitzin is also a primary parasitoid of *P. sinica* when it was first recorded in 2016 from China. To assess the effect of *M. picta* on the local biological control system, its type and biological characteristics were studied under laboratory conditions, and the levels of hyperparasitism were estimated from 2015 to 2017 in the field. The laboratory results showed that *M. picta* was an obligate solitary hyperparasitoid. It preferred to attack the mature larvae of *T. lyciuni* but was not able to attack *P. arenarius*. Field surveys suggested that introduction of *M. picta* reduced the second wave of parasitism by *T. lyciuni*. Free from the hyperparasitism, the well-timed presence of *P. arenarius* adequately compensated for this hyperparasitoid-driven loss, significantly decreasing the population of *P. sinica*. Because of the mediation by *M. picta*, the newcomer *P. arenarius* could successfully establish a population and co-exist with *T. lyciuni* to synergistically suppress the populations of *P. sinica*.

Keywords: *Paratrioza sinica*, *Tamarixia lyciuni*, *Psyllaephagus arenarius*, biological control, synergistic effect

1 Introduction

Pest infestations are conspicuous, but the arrival of parasitoids is often neglected due to small size. However parasitoids are likely to have significant effects on pest suppression once they are intentionally or accidentally introduced (Murray & Mansfield 2015). Accidental introductions of primary parasitoids are common (Charles 1993, Johnson et al. 2001, Calcaterra et al. 2007, Bjørnson 2008), but hyperparasitoids often receive less attention than primary parasitoids (Yang et al. 2017). Despite relatively limited studies on accidental introductions of hyperparasitoids, their effects on pest suppression are far greater than reported (Wang & Messing 2004, Peck et al. 2008). Some studies suggest hyperparasitoids may disrupt biological control by influencing the efficiency of primary parasitoids (Yang et al. 2017, Gomez-Marco et al. 2015, Schooler et al. 2011). However, others indicate that hyperparasitoids can facilitate the coexistence of primary parasitoids by stabilizing host-parasitoid and

parasitoid-parasitoid dynamics (Sullivan 1987, Hassell & Waage 1984, Harvey et al. 2003). As an important component of the fourth trophic level, hyperparasitoids often damage the efficiency of primary parasitoids by reducing the population of the biocontrol agents (Poelman et al. 2012, Bain & Kay 1989, Höller et al. 1993). Therefore, the standard of biological control programme is to consider primary parasitoids less susceptible to hyperparasitism in the local area (Berry & Mansfield 2006).

Paratrioza sinica Yang et Li (Homoptera: Psyllidae), a harmful leaf-sucking pest of *Lycium barbarum* L., is widespread across goji berry orchards in northern China (Wang et al. 2010, Liu et al. 2013, Wu et al. 2017). Currently, broad-spectrum chemical pesticides are used to control *P. sinica*, potentially damaging human health and the ecological environment (Youn et al. 2003, Garratt & Kennedy 2006). Biological control programs would be more sustainable and desirable, thus they are gaining increasing attention (Leskey et al. 2012, Lee et al. 2013). Parasitoids have

a potential to create tremendous ecological and economic values for biological control programs (Hawkins et al. 1999). *Tamarixia lyciuni* Yang (Hymenoptera: Eulophidae) and *Psyllaephagus arenarius* Trjapitzin (Hymenoptera: Encyrtidae) are primary parasitoids of *P. sinica*. Native to the region, *T. lyciuni* was originally recorded in 1997 and has been an efficient biocontrol agent of *P. sinica* in China (Tang 1997, Duan et al. 2002). As an ectoparasitoid, *T. lyciuni* glues its egg externally to the host and hatched larva feeds externally on the host till it pupates. From 2016, the hyperparasitoid *Marietta picta* Andre (Hymenoptera: Aphelinidae) was first found in Ningxia to frequently attack *T. lyciuni* in the field. Simultaneously, self-introduced *P. arenarius* was also first described in 2016 from the same location (Zhang et al. 2017). *Psyllaephagus arenarius* is an endoparasitoid, it deposits eggs inside the host, in which they continue to grow as larvae, allowing the development of their host. Because of high parasitism rate, *P. arenarius* has a great chance to establish a population in the local area and acts as an alternative biocontrol agent of *P. sinica* (Wu et al. 2017, 2018).

Currently, *T. lyciuni* was frequently hyperparasitized by *M. picta* in the field. But it remained unclear whether *P. arenarius* was also hyperparasitized, and whether *M. picta* was an obligate or facultative hyperparasitoid. Thus, in this study, we aimed to determine the type (obligate or facultative hyperparasitoid) and biological characteristics (hyperparasitism rate, emergence rate, female parasitoid rate and pre-emergence time) of *M. picta* under laboratory conditions, and most importantly, to assess the levels of hyperparasitism of *M. picta* in the field from 2015 to 2017 and its effect on the local biological control system.

2 Materials and methods

2.1 Insects

Fresh *P. sinica* nymphs were collected from a large goji berry orchard (length 120 m × width 35 m) in Dadi of the Ningxia Province, China (GPS: N37°27', E105°47'). Colonies of *T. lyciuni* and *P. arenarius* were established from parasitized *P. sinica* nymphs in the Dadi goji berry orchard, and both species of *T. lyciuni* (Wang et al. 2010) and *P. arenarius* (Zhang et al. 2017) were taxonomically identified via morphology. The colony of *M. picta* was established from *T. lyciuni* mummies collected from the same orchard. Upon the initial establishment of the laboratory colony, the specimen of *M. picta* was taxonomically identified by Y. Zhang (Institute of Zoology, Chinese Academy of Sciences, Beijing, China). Colonies of the parasitoids and hyperparasitoid were maintained in incubators (MKF240, Binder, Tuttlingen, Germany) at 26 ± 1 °C, 70 ± 5% RH, 16L:8D photoperiod and were reared for over six generations. Host feeding of *M. picta* was not observed. Adult *M. picta* were provided with additional 10% honey water twice a week besides

T. lyciuni mummies. All parasitoid females used in laboratory experiments were 4-d old and mated. The experiments were performed at 26 ± 1 °C, 70 ± 5% RH, 16L:8D photoperiod.

2.2 Type and biological characteristics of *M. picta*

Since both *T. lyciuni* and *P. arenarius* showed remarkable preferences for 4th-instar nymphs of *P. sinica* with high parasitism rates (Wang et al. 2010, Wu et al. 2017), this stage was selected as the host for both primary parasitoids. To test the hyperparasitism by *M. picta*, 20 fresh (< 12 h) 4th-instar nymphs of *P. sinica* were firstly exposed to randomly selected *T. lyciuni* females in a Petri dish (9 cm diameter, YH253-320, Brand, Wertheim, Germany). Zero, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 days after being parasitized by *T. lyciuni*, the 20 hosts parasitized by *T. lyciuni* were offered to a *M. picta* female in the Petri dish. A similar protocol was used with the primary parasitoid *P. arenarius*, 20 fresh 4th-instar nymphs of *P. sinica* were exposed to *P. arenarius* females, and the 20 hosts parasitized by *P. arenarius* were subsequently exposed to a *M. picta* female 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 days later. In addition, 20 *P. sinica* individuals of each stage (egg or, 1st-, 2nd-, 3rd-, 4th- or 5th-instar nymph) were offered to a *M. picta* female in a Petri dish to determine obligate or facultative hyperparasitism. The handling time of *M. picta* was about 5.3 min per host, the petri dish was monitored until the *M. picta* female stopped interacting with the hosts for at least 30 min. After 24 h, the *M. picta* female was removed, and the Petri dish was kept at the same conditions to allow the development of larval parasitoids and hyperparasitoids. The oviposition by *M. picta* could be identified by an obvious parasitization event and the presence of a distinctive scar on the primary parasitoid. To examine the parasitism characteristics of *M. picta*, the number of primary parasitoids hyperparasitized by *M. picta* was counted to calculate the proportion (hyperparasitism rate). Then the Petri dish was checked daily until no parasitoids and hyperparasitoids emerged at all. After three weeks, the proportion of *M. picta* emerging from hyperparasitized primary parasitoids (emergence rate), and the female proportion in the emerging hyperparasitoids (female parasitoid rate) were recorded. Species and sex of the emerging hyperparasitoids were identified based on morphological characteristics. Moreover, the developmental time of *M. picta* from oviposition to adult emergence (pre-emergence time) was examined. The experiment was replicated 10 times simultaneously.

2.3 Parasitism and hyperparasitism in the field

The field surveys were conducted from 2015 to 2017 at the IPM demonstration plots located in the Dadi goji berry orchard. To estimate the levels of hyperparasitism by *M. picta* in the field, a goji berry plant was randomly selected. Overall 15 leaves were collected from 5 directions (east, south, west,

north, and center part of a plant) and from 3 layers (upper, middle, and lower third of a plant). *Tamarixia lyciumi* and *P. arenarius* merely parasitized 3rd–5th instar nymphs of *P. sinica*. Thus, the number of 3rd–5th instar nymphs of *P. sinica* per leaf, the parasitism rates of *T. lyciumi* and *P. arenarius*, and the hyperparasitism rate of *M. picta* were calculated:

Parasitism rates (%) = [(number of 3rd–5th instar nymphs of *P. sinica* parasitized) / (number of 3rd–5th instar nymphs of *P. sinica*)] × 100

Hyperparasitism rate (%) = [(number of primary parasitoids hyperparasitized by *M. picta*) / (number of primary parasitoids)] × 100

Sampling started after parasitoids emerged, once per week from May to October of each year during the emergence of parasitoids. Each sampling was replicated 10 times simultaneously.

2.4 Data analysis

Descriptive statistics were given as the mean values and standard errors of the mean. Proportions of *T. lyciumi* at different ages hyperparasitized by *M. picta* were analysed with χ^2 tests. The value for each combination of factors was calculated according to the resulting standardized residual via crosstab analysis, and compared to Bonferroni-corrected *P* values testing whether each hyperparasitism rate was significantly different from the average rate across parasitoid ages. Other data were analyzed using independent-samples T test, *P* values < 0.05 were considered significant. All statistical analyses were performed using SPSS 20.0 software (IBM, Armonk, NY).

3 Results

3.1 Type and biological characteristics of *M. picta*

Marietta picta did not parasitize *P. sinica* regardless of stage and instar, and it also did not hyperparasitize the primary parasitoid *P. arenarius* of any age. However, *M. picta* showed significantly positive preference for the mature larvae of *T. lyciumi* (5–6 days old), while the eggs and younger larvae (0–2 days old), and older pupae (11–14 days old) were less acceptable ($\chi^2_{(14, N=3000)} = 545.029, P < 0.0001$; 30 comparison tests, adjusted $\alpha = 0.001667$; Fig. 1).

Only one *M. picta* egg was deposited and then only one *M. picta* adult had a possibility of emerging from a *T. lyciumi* individual. The average hyperparasitism rate of *M. picta* was 14.8±2.9%. It mainly hyperparasitized the mature larvae of *T. lyciumi*, and the hyperparasitism rate reached occasionally up to 36.0±4.8% on the 5-d-old (Fig. 1). On the hyperparasitized *T. lyciumi*, the emergence rate of *M. picta* was 46.6±2.6%, and 56.6±3.2% of the emerging *M. picta* were females. *Marietta picta* had a pre-emergence time of 14.45±2.91 days including 1.75±0.43, 4.17±0.74, 1.62±0.31 and 6.91±1.64 days for the egg, larval, prepupal and pupal stages, respectively.

3.2 Parasitism and hyperparasitism in the field

3.2.1 Year 2015

In 2015 *T. lyciumi* was the only parasitoid in the goji berry orchard, the primary parasitoid *T. lyciumi* started parasitizing the 3rd–5th instar nymphs of *P. sinica* at the end of May,

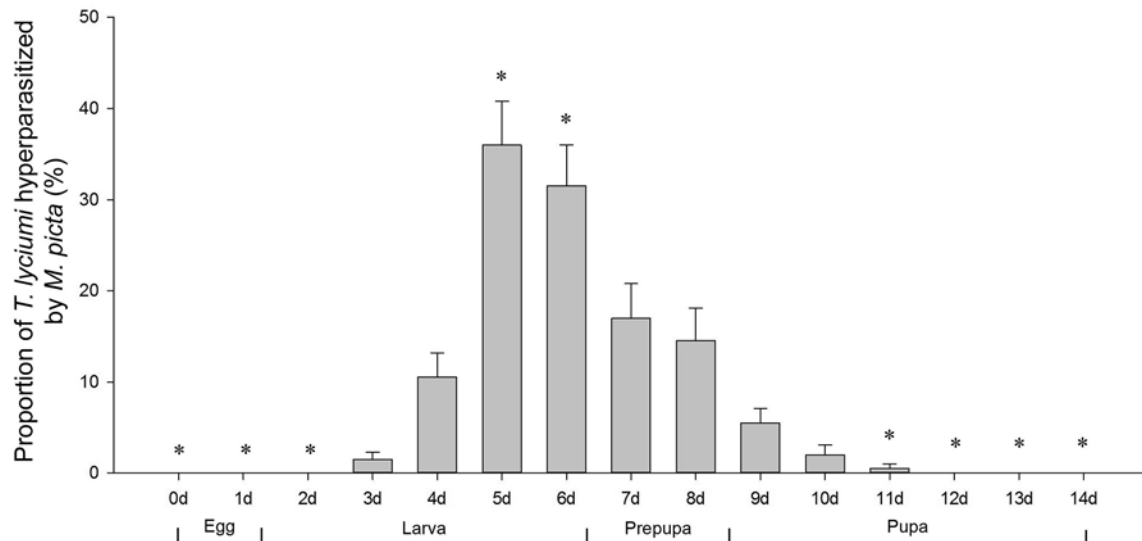


Fig. 1. Proportions of *Tamarixia lyciumi* at different ages (d) hyperparasitized by *Marietta picta* (Means ± SE). Asterisks (*) indicate proportions of hyperparasitized *T. lyciumi* differ significantly from the mean expected proportion across the parasitoid ages, according to χ^2 tests with Bonferroni corrections.

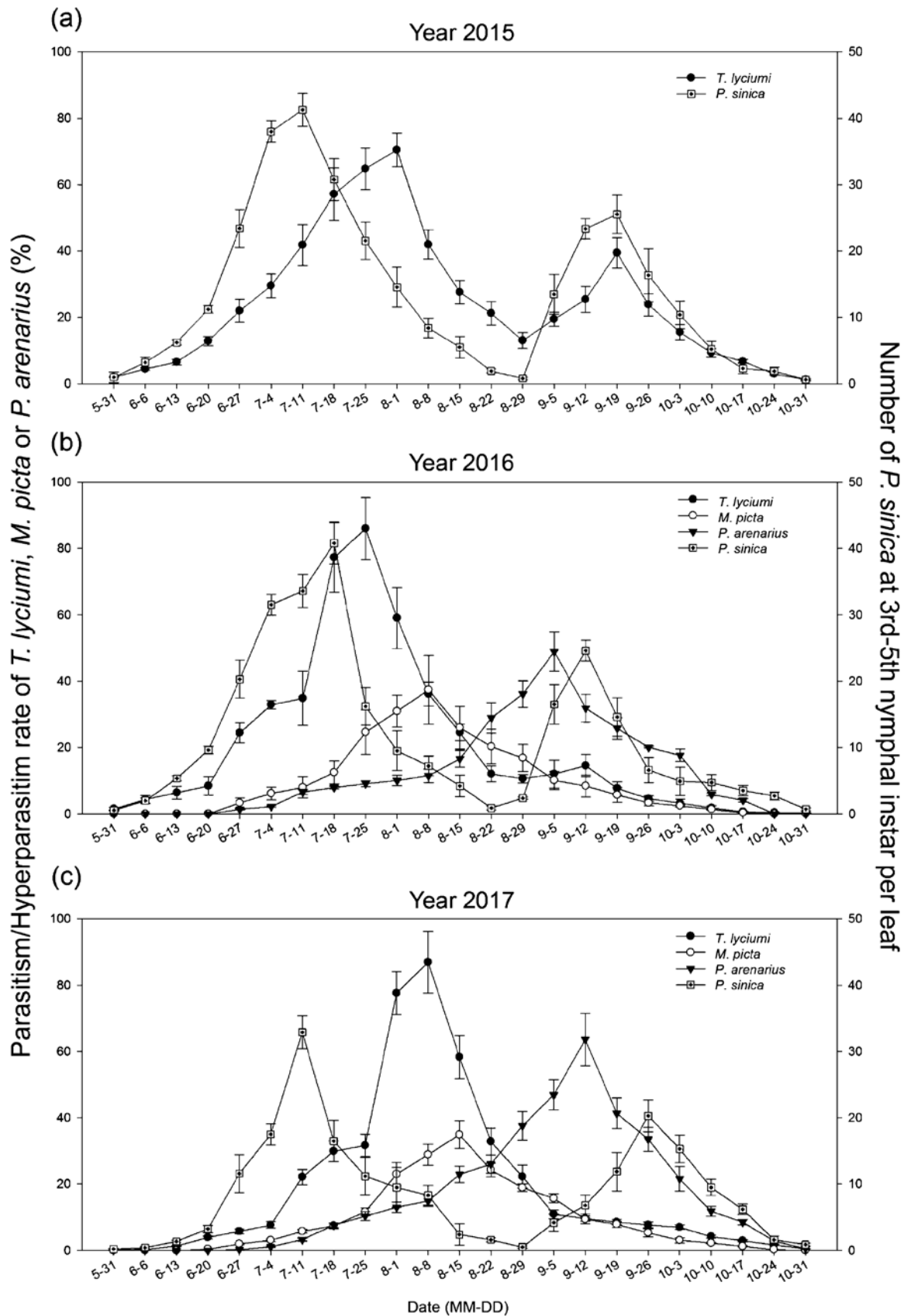


Fig. 2. Evolution of the host-parasitoid-hyperparasitoid system from 2015 to 2017 in the goji berry orchard in Ningxia (a, year 2015; b, year 2016; c, year 2017). Primary vertical axis on the left: Percentage of parasitism by *Tamarixia lyciumi* or *Psyllaephagus arenarius*, and percentage of hyperparasitism by *Marietta picta*. Secondary vertical axis on the right: Number of 3rd–5th instar nymphs of the host *Paratrioza sinica* per leaf. Values are mean \pm SE.

during the second generation of *P. sinica*. The parasitism rate of *T. lyciuni* increased rapidly in Jun and Jul, reaching a peak on 1 Aug (70.5±5.1%). After this peak, the parasitism rate decreased to 13.0±2.4% on 29 Aug, and then increased again during the early Sep. The second peak of parasitism rate occurred on 19 Sep (39.4±4.6%), and in the late Sep and in Oct the parasitism gradually vanished due to lack of host (Fig. 2a). During this period, the average number of the host nymphs was 13.3±2.6 per leaf. In terms of parasitism, there were two waves occurring before and after 29 Aug, and the average parasitism rates were 29.7±6.0% and 15.7±3.7%, respectively. The overall parasitism rate of *T. lyciuni* in 2015 was 24.3±4.2%.

3.2.2 Year 2016

From a *T. lyciuni* perspective, the parasitism rate increased rapidly in the late Jun and in Jul, reaching a peak on 25 Jul (86.0±9.4%). Meanwhile, the *M. picta* population started on 20 Jun 2016 and showed a peak of hyperparasitism on 8 Aug reaching 37.4±10.4%, the peak in the hyperparasitism rate of *M. picta* lagged 2 weeks following the parasitism peak of *T. lyciuni*. After the peak, the parasitism level of *T. lyciuni* steadily decreased, although a slight build-up of parasitism was detected on 12 Sep (14.5±3.3%), the so-called second peak was obviously lower than the first one. Moreover, the newcomer *P. arenarius* was detected in late Jun 2016 and its parasitism showed a peak on 5 Sep reaching 48.9±5.9% (Fig. 2b).

From a *T. lyciuni* perspective, the first wave of parasitism (before 29 Aug) did not undergo a change between 2015 and 2016 (year 2015, 29.7±6.0%; year 2016, 29.9±7.2%; $t=0.02$, $df=26$, $P=0.984$), whereas the second wave was largely suppressed (year 2015, 15.7±3.7%; year 2016, 5.6±1.7%; $t=2.49$, $df=18$, $P=0.023$). Overall, despite the emergence of *M. picta* in 2016 (the average hyperparasitism rate was 9.4±2.5%), the average parasitism rate of *T. lyciuni* was not significantly impacted (year 2015, 24.3±4.2%; year 2016, 20.1±3.1%; $t=0.547$, $df=44$, $P=0.587$), and the well-timed presence of *P. arenarius* adequately compensated for the hyperparasitoid-driven loss. Since the newcomer *P. arenarius* (the average parasitism rate was 12.4±1.5%) supplemented the second wave of parasitism by *T. lyciuni*, the average population of the host nymphs in 2016 (11.4±2.4 per leaf) still decreased compared to 2015 (13.3±2.6 per leaf) ($t=0.547$, $df=44$, $P=0.587$).

3.2.3 Year 2017

Marietta picta showed a peak of hyperparasitism on 15 Aug 2017 (34.9±4.2%), no difference was detected in average hyperparasitism rates between 2016 (9.4±2.5%) and 2017 (8.9±2.2%) ($t=0.162$, $df=44$, $P=0.872$). Because of continuous hyperparasitism by *M. picta*, only the first wave of parasitism by *T. lyciuni* was detected, the second wave disappeared totally, the only peak of parasitism occurred on 8 Aug (86.9±9.3%). Fortunately, *P. arenarius* completely

filled the vacancy of second wave of parasitism by *T. lyciuni*. The peak of parasitism by *P. arenarius* (63.6±7.8%) occurred on 12 Sep, almost consistent with the time of second peak of parasitism by *T. lyciuni* in 2015 (19 Sep) or 2016 (12 Sep) (Fig. 2c).

After continuous hyperparasitism by *M. picta*, the average parasitism rate of *T. lyciuni* in 2017 (18.9±5.1%) decreased slightly compared to 2016 (20.1±5.1%) ($t=0.176$, $df=44$, $P=0.861$), whereas that of *P. arenarius* greatly increased from 2016 (12.4±2.9%) to 2017 (22.9±4.4%) ($t=2.099$, $df=44$, $P=0.043$). Since the newcomer *P. arenarius* further supplemented the second wave of parasitism by *T. lyciuni*, the average population of the host nymphs in 2017 (5.7±1.2 per leaf) significantly decreased compared to 2016 (11.4±2.4 per leaf) ($t=2.054$, $df=32.015$, $P=0.048$).

4 Discussion

After accidental introductions of a hyperparasitoid, its effect (neutral, positive or negative) on biological control may depend on its type (obligate or facultative hyperparasitoids) and biological characteristics (Murray & Mansfield 2015). From a pest suppression perspective, it is necessary to minimize the damage that a hyperparasitoid could cause (Bistline-East & Hoddle 2016). Hyperparasitoids with a hyperparasitism rate higher than the emergence rate of their parasitoid hosts could greatly impact biological control of a pest (Schooler et al. 2011). It is thus important to assess whether the hyperparasitism below the level necessary to control the pest. *Marietta picta* can discriminate its host (i.e. *T. lyciuni*) previously parasitized by self or conspecifics to avoid superparasitism. It only glues one egg externally to *T. lyciuni* larva, and a larval *M. picta* hatches and feeds externally on the host until pupation, which is the defining characteristic of solitary ectoparasitoid (Asgari & Rivers 2011). Moreover, *M. picta* failed to develop on the herbivore pest *P. sinica* and the primary parasitoid *P. arenarius*, merely hyperparasitized *T. lyciuni* (especially the mature larvae), suggesting that *M. picta* is an obligate solitary hyperparasitoid in the local host-parasitoid-hyperparasitoid system.

Not only the type but also the biological characteristics of a hyperparasitoid could impact the efficacy of biological control. For example, the hyperparasitoid *Asaphes suspensus* with higher hyperparasitism rate can lead to the extinction of primary aphid parasitoid *Aphidius ervi*, thereby causing overpopulation of the pea aphid *Acyrtosiphon pisum* (Schooler et al. 2011). In terms of hyperparasitoids, hyperparasitism rate, emergence rate, female parasitoid rate and pre-emergence time are important parameters, and these biological characteristics determine the effect of hyperparasitoid on pest suppression. From a biological control viewpoint, the hyperparasitism rate of hyperparasitoids is expected to be inferior to the emergence rate of their parasitoid hosts (Sullivan & Völkl 1999). Even if the hyperparasitism rate of

M. picta could reach occasionally up to $36.0 \pm 4.8\%$ in laboratory situations, it was still far lower than the emergence rate of *T. lyciumi* ($87.33 \pm 1.15\%$; Liu et al. 2010). The data suggest that to a large extent *M. picta* is unlikely to result in the local extinction of *T. lyciumi*. However, *M. picta* may disrupt the native biological control by threatening the population of *T. lyciumi*.

Some studies suggest, although hyperparasitoid fails to cause extinction of parasitoids, it can still disrupt biological control systems (May & Hassell 1981). After accidental introductions of hyperparasitoids, the primary parasitoids *Enoggera nassau* (Murphy & Kay 2000) and *Enoggera reticulata* (Tribe 2000) cannot successfully control their host pest, implying hyperparasitoids can disrupt biological control by exerting considerable pressure on primary parasitoids (de Little 1982). However, others indicate many primary parasitoids still remain economically successful in the field, despite their poor performances under hyperparasitism conditions in laboratory tests (Hammond & Neuenschwander 1990, Gaines & Kok 1999, Day 2002, Wang & Messing 2004). Laboratory outcomes cannot be directly extrapolated to results of field surveys as laboratory tests are simplified systems (Bográn et al. 2002). High levels of hyperparasitism alone do not reliably indicate it can disrupt biological control systems (Rosenheim 1998). In fact, our field studies showed that the average hyperparasitism rate of *M. picta* ($9.2 \pm 2.4\%$) in open fields was far lower than the result of laboratory research ($14.8 \pm 2.9\%$), and the population of *T. lyciumi* did not significantly decrease, suggesting *M. picta* may not necessarily disrupt the native biological control. However, hyperparasitoids may potentially weaken biological control systems by continuously decreasing populations of primary parasitoids (Schooler et al. 2011), and they may even cause a male-biased sex ratio of primary parasitoids (Gomez-Márco et al. 2015). Thus, it is necessary for biological control programme to consider an alternative biocontrol agent of goji berry psyllid in advance. Our results verified that the primary parasitoid *P. arenarius* was not hyperparasitized by *M. picta*. Thus, the chance of establishment of self-introduced *P. arenarius* in local area and its interactions with native *T. lyciumi* deserve to be evaluated.

According to results in the field, *T. lyciumi* was the only biocontrol agent of *P. sinica* in Ningxia before 2015. From 2016, the population of *T. lyciumi* was threatened by the accidentally introduced hyperparasitoid *M. picta*. Even if obligate hyperparasitoids do not greatly affect the overall efficiency of pest suppression (Jones & Withers 2003), the heavy summer hyperparasitism significantly reduces the density of parasitoid host going into winter (Murray & Mansfield 2015). Thus, the second wave of parasitism by *T. lyciumi* was largely suppressed in 2016, probably due to the heavy summer hyperparasitism by *M. picta*. Interestingly, the presence of self-introduced *P. arenarius* was well-timed, it was also first recorded from Ningxia in 2016 (Zhang et al. 2017). Free from the hyperparasitism, *P. arenarius* could

adequately compensate for the hyperparasitoid-driven loss. The peak of parasitism by *P. arenarius* occurred in early Sep, almost consistent with the time of the second peak of parasitism by *T. lyciumi*. Because of continuous hyperparasitism by *M. picta* from 2016 to 2017, the second wave of parasitism by *T. lyciumi* disappeared totally in 2017, and *P. arenarius* completely filled the vacancy. The parasitism rate of *P. arenarius* ($22.9 \pm 4.4\%$) was even higher than that of *T. lyciumi* ($18.9 \pm 5.1\%$) in 2017, suggesting the newcomer *P. arenarius* can establish a population in Ningxia. Thus, *P. arenarius* started to show its potential of parasitism in 2017 and worked synergistically with *T. lyciumi* to compensate for the hyperparasitoid-driven loss in native biological control. The population of *P. sinica* significantly decreased from 2016 to 2017, indicating the synergistic effect of both parasitoid species greatly improve the pest suppression.

In many cases, hyperparasitoids have stabilizing effects by mediating parasitoid-parasitoid interactions to improve pest suppression (Beddington & Hammond 1977, Sullivan 1987). Thanks to the hyperparasitism by *M. picta*, the newcomer *P. arenarius* has a chance to compensate for the hyperparasitoid-driven loss by efficiently complementing the second wave of parasitism by native *T. lyciumi*, and to successfully establish its population in Ningxia. There is no overlap (5-week interval) between the parasitism peaks of *P. arenarius* and *T. lyciumi* due to the mediation by *M. picta*, maximizing the synergistic effect of both parasitoid species in controlling *P. sinica*. Instead of having negative effects, the hyperparasitoid *M. picta* mediates the coexistence of parasitoids in the local area, contributing greatly to the synergistic effect of various biocontrol agents in pest suppression.

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