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Exploration for native parasitoids of *Drosophila suzukii* in China reveals a diversity of parasitoid species and narrow host range of the dominant parasitoid

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Abstract

Drosophila suzukii is native to East Asia and an invasive pest of fruit crops widely established in the Americas and Europe. The lack of effective indigenous parasitoids of *D. suzukii* in the invaded regions prompted surveys for co-evolved parasitoids in Yunnan Province, China, from 2013 to 2016. From banana-baited traps (2013–2015), 458 parasitoids of drosophilids were reared, comprised of Braconidae (49.56%), Figitidae (37.55%), Diapriidae (7.42%), and Pteromalidae (5.46%). Larval parasitoids included seven braconid species, all *Asobara* and primarily *Asobara mesocauda*, and five figitid species, primarily *Leptopilina japonica japonica*. Pupal parasitoids were the diapriid *Trichopria drosophilae* and the pteromalid *Pachycrepoideus vindemiae*. Collections from wild fruits (2016) provided more interesting results. From the puparia of drosophilids collected, comprised of *D. suzukii* and *Drosophila pulchrella*, emerged 1354 parasitoids. The larval parasitoids *Ganaspis brasiliensis* and *L. j. japonica* were the prevalent species, reaching a fairly high percentage parasitism of fly puparia collected from berries of *Rubus foliosus* (22.35%), *R. niveus* (18.81%), *Fragaria moupinensis* (19.75%), and *Sambucus adnata* (63.46%). *Ganaspis brasiliensis* was the dominant species and was collected only from *D. suzukii* and *D. pulchrella*-infested fruits and never from banana-baited traps. Molecular analysis showed two *G. brasiliensis* lineages, which are discussed with respect to previous Japanese collections. Quarantine tests showed that *G. brasiliensis* developed from *D. suzukii* and two closely related hosts (*Drosophila melanogaster* and *Drosophila simulans*) but did not develop from seven non-target drosophilid species. Our results suggest that *G. brasiliensis* is a promising classical biocontrol agent for release in invaded regions.

Keywords Classical biological control · *Ganaspis brasiliensis* · Genetic variation · *Leptopilina japonica japonica* · Invasive species · Spotted wing drosophila

Key message

- *Drosophila suzukii* is an invasive pest without effective natural enemies in invaded regions.
- Surveys for native parasitoids were conducted from 2013 to 2016 in China.
- 458 (15 species) and 1354 (6 species) parasitoids were reared from drosophilids in fruit-baited traps, or *D. suzukii* and *Drosophila pulchrella* developing in field-collected fruits, respectively.
- *Ganaspis brasiliensis* was the dominant species recovered only from field-collected fruits.
- The narrow host range showed by *G. brasiliensis* both in the field and in the laboratory makes this species a potential agent for classical biological control.

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Introduction

The spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is native to the Eastern and Southeastern regions of Asia but has become a serious invasive pest causing considerable economic damage to fruit crops worldwide (Asplen et al. 2015). Current management of *D. suzukii* in Europe or Americas relies primarily on insecticide applications (Beers et al. 2011; Van Timmeren and Isaacs 2013). However, this approach is often ineffective in preventing all economic damage, in part because there is often a diversity of wild flora neighboring cultivated fields that can serve as refugia for the pest to recolonizing treated crops (Kenis et al. 2016; Klick et al. 2016; Lee et al. 2015). Therefore, there is a critical need to develop additional control tools for this extremely polyphagous and mobile pest, and to include area-wide management strategies that can suppress populations in the entire landscape (Haye et al. 2016; Wang et al. 2016b). From this perspective, classical biological control through the introduction of Asian natural enemies could play a key role in reducing *D. suzukii* populations in non-cultivated habitats.

A complex of larval and pupal parasitoids plays an important role in the natural suppression of many drosophilid species (Carton et al. 1986), with levels of parasitism often exceeding 50% (Fleury et al. 2009). Unfortunately, for *D. suzukii* in North America and Europe, most resident larval parasitoids are unable to complete development because of *D. suzukii*'s stronger cellular immune response than native Drosophilidae species (Chabert et al. 2012; Kacsoh and Schlenke 2012; Poyet et al. 2013). Only a few generalist indigenous parasitoid species such as the pupal parasitoids *Trichopria drosophilae* (Perkins) (Hymenoptera: Diapriidae) and *Pachycrepoideus vindemiae* (Rondani) (Hymenoptera: Pteromalidae) have been reported to readily attack *D. suzukii* in both Europe (Gabarra et al. 2015; Rossi Stacconi et al. 2013, Mazzetto et al. 2016, Knoll et al. 2017) and North America (Miller et al. 2015; Wang et al. 2016a, b). A strain of the larval parasitoid *Leptopilina heterotoma* (Thomson) (Hymenoptera: Figitidae) in northern Italy has been reported to attack *D. suzukii* in the laboratory but does not provide adequate control levels (Rossi Stacconi et al. 2015, 2017), while most tested *L. heterotoma* populations failed to develop from *D. suzukii* (Chabert et al. 2012; Miller et al. 2015; Mazzetto et al. 2016; Knoll et al. 2017). Overall, extant parasitoids have not provided sufficient control of *D. suzukii* in invaded regions (Gabarra et al. 2015; Miller et al. 2015; Rossi Stacconi et al. 2015; Wiman et al. 2016).

The lack of effective natural enemies of *D. suzukii* in Europe and North America led to recent field surveys in Asia for native, co-evolved and specialized parasitoids to be considered for use in classical biological control programs (Daane et al. 2016; Guerrieri et al. 2016; Girod et al. 2018a; Giorgini et al., reported herein). Previously, *D. suzukii*–parasitoid associations were described in Japan, with 10 parasitoid species reported (Ideo et al. 2008; Mitsui and Kimura 2010; Mitsui et al. 2007) among which they identified a 'strain' of *Ganaspis xanthopoda* (Hymenoptera: Figitidae) being the most active parasitoid and considered a specialist of *D. suzukii* (Kasuya et al. 2013). Additionally, as a point of clarification, we note that *Ganaspis* individuals recorded from *D. suzukii* and previously reported as *G. xanthopoda* by Kasuya et al. (2013), Mitsui et al. (2007) and Mitsui and Kimura (2010) have been assigned the name *Ganaspis brasiliensis* (Ihering) by Buffington and Forshage (2016) in their treatment of *Ganaspis* associated with *D. suzukii*. This updated name was utilized by Nomano et al. (2017) who grouped *G. brasiliensis* into five genetic lineages, with just two recorded from *D. suzukii*. The populations of *Ganaspis* found to parasitize *D. suzukii* in China and Japan by Girod et al. (2018a, b, c) are also morphologically attributable to *G. brasiliensis*.

We previously reported on the presence and identity of parasitoid species in the genus *Asobara* (Hymenoptera: Braconidae), collected from Yunnan Province, China, that were associated with drosophilids; seven parasitoid species were identified including five new species (Guerrieri et al. 2016). In South Korean collections, six larval parasitoid species (*Asobara brevicauda* van Achterberg and Guerrieri, *A. japonica*, *A. leveri* (Nixon), *L. j. japonica*, *L. japonica formosana* Novković and Kimura, and *G. brasiliensis*) and one pupal parasitoid (*T. drosophilae*) were recorded from *D. suzukii* (Daane et al. 2016). Interestingly, *A. brevicauda*, *L. j. japonica*, and *G. brasiliensis* emerged only from *D. suzukii* from field-collected fruits but not from traps baited with uninfested fruit (which yielded predominately other *Drosophila* species) (Daane et al. 2016). To complement these recent explorations and to look for parasitoid species with potentially higher efficacy and specificity to *D. suzukii*, we conducted surveys in the Yunnan Province, China, from 2013 to 2016. Here, we report on (1) parasitoid species collected using banana-baited traps and through direct sampling of *D. suzukii* host plant fruits; (2) genetic analyses of *G. brasiliensis* collected; and (3) quarantine test of host specificity of *G. brasiliensis* with 10 different drosophila species. Our findings in Yunnan expand the current knowledge on the diversity, geographic distribution and host range of parasitoid species attacking drosophilids, and specifically *D. suzukii* in Asia.

Materials and methods

Parasitoid collections

Surveys for *D. suzukii* parasitoids were conducted in summers from 2013 to 2016 in different locations of Yunnan Province, China (Table 1), using either banana-baited traps placed near natural vegetation or cultivated fields, or collections of fruits from natural vegetation including known or presumed host plants of *D. suzukii*. This region in southern China is part of the presumptive native range of *D. suzukii* and the closely related species *Drosophila pulchrella* Tan, Hsu & Sheng (Takamori et al. 2006; Zhao et al. 2017), both characterized by a serrated ovipositor that allows them to penetrate the intact skin of fruits.

Banana-baited traps were used from 2013 to 2015. The traps were made of plastic food boxes (10 × 15 × 30 cm) with 0.5-cm holes along the side for ventilation and provisioned with sliced sections of banana for fruit fly egg deposition (developing into fresh larvae available for parasitization). At each of the seven sampled sites, 4–11 traps were placed in a linear transect at distances of ~ 100 m from each other. After 7 days, traps were collected and transferred to a laboratory (Yunnan Academy of Agricultural Science), where the ventilation holes were covered with organdy and the traps were held at 25 ± 3 °C and then observed daily for fly or parasitoid emergence. Emerged flies and parasitoids were collected and immediately killed in absolute ethanol and preserved at − 20 °C for subsequent species identification.

Field collection of fruits was adopted in 2015 (limited collections) and 2016 to get more precise information on the

Table 1 Sampling locations for drosophilid parasitoids and collection methods during 2013–2016 in Yunnan Province, China

Location	Coordinates	Altitude (m)	Sampling date	Sampling method (no. of traps), habitat or fruit
1. Long Jie Zuo Suo Cun, Cheng Jiang County	24.711991N 102.870912E	2053	2013.vii.17	Banana traps (9), wild vegetation
2. Fu Xian Lake, Cheng Jiang County	24.506364N 102.860508E	1759	2013.vii.17	Banana traps (8), wild vegetation surrounding blueberry cultivations
3. Kang Yi Zu Resort, Shi Lin County	24.900200N 103.364400E	1854	2013.vii.13	Banana traps (8), surrounding blueberry cultivations
4. Stone Forest Scenic Area, Shi Lin County	24.815151N 103.322628E	1793	2015.x.3	*Osyris wightiana* berries (no isolation of puparia)
5. Kunming Botanical Gardens, Pan Long District, Kunming	25.145348N 102.741543E	1958	2013.vii.15	Banana traps (7) in a public botanical garden
6. Xiao He Research Farm, Pan Long District, Kunming	25.194913N 102.805263E	2171	2013.vii.15	Banana traps (8), wild vegetation near orchards
7. Xiao He Research Farm Pan Long District, Kunming	25.171488N 102.780428E	1996	2013.vii.16	Banana traps (11), wild vegetation
8. Xi Shan Forest, Xi Shan District, Kunming	24.984073N 102.622356E	1950	2013.vii.18	Banana traps (11), unspoiled wild vegetation
7. Xiao He Research Farm, Pan Long District, Kunming	25.176593N 102.794697E	2209	2014.vii.15 2014.viii.15 2014.x.15 2015.v.20 2015.vi.20 2015.vii.20 2015.viii.20	Banana traps (4), wild vegetation near orchards
9. Liang Wang Shan, Cheng Jiang County	24.765831N 102.889780E	2452	2016.vii.13	Puparia collection from *Rubus niveus* berries
10. Chang Chong Shan, Wu Hua District, Kunming	25.132223N 102.706662E	2207	2016.vii.6 2016.vii.12 2016.vii.19	Puparia collection from *Rubus niveus* and *R. foliosus* berries
11. Dong Da Cun, Pan Long District, Kunming	25.098602N 102.835000E	2239	2016.vii.6 2016.vii.8 2016.vii.16 2016.vii.18	Puparia collection from *Fragaria moupinensis*, *Rubus foliosus* and *Sambucus adnata* berries
			2016.vii.25	Puparia collection from *Sambucus adnata* berries
12. Lei Da Shi Cun, Pan Long District, Kunming	24.748332N 102.881391E	2532	2016.vii.11	Puparia collection from *Rubus niveus* berries

association among host plant-*Drosophila*-parasitoid species, and on the level of field parasitism. Fruits were collected in July 2016 from plants of *Sambucus adnata* Wallich ex de Candolle, *Rubus foliosus* Weihe, *Rubus niveus* Thunberg, and *Fragaria moupinensis* Cardot (Table 1) and taken to a laboratory (Yunnan Academy of Agricultural Science) where they were stored at 25 ± 3 °C in plastic boxes, as described previously, and sorted by host plant, collection date and site. The fruit were checked daily under a binocular microscope (60×) for a period of 7–10 days after field collection for *Drosophila* puparia. When discovered, the *D. suzukii* and *D. pulchrella* puparia were separated from those of other drosophilids by the characteristic shape of the two everted anterior spiracles (tubes with finger-like projections). It was not possible, however, to sort the puparia of *D. suzukii* from those of *D. pulchrella*, based on puparium morphology (Hauser 2011). For this reason, we refer to collected puparia of these two fly species as *D. suzukii*-like puparia. These latter represented almost all (~ 99%) puparia found in collected fruits. Groups of 10–20 collected puparia were isolated in glass vials (1 × 7.5 cm) that were sealed with a cotton plug and provisioned with moistened filter paper strip to prevent desiccation (but trying to avoid water condensation or deposition). Adult fly and parasitoid emergence was checked every 2–3 days for a period of 1 month (either at the laboratory of the Yunnan Academy of Agricultural Science or in quarantines at the University of California, Berkeley, USA, and at USDA Agricultural Research Service, Beneficial Insects Introduction Research Unit, Newark, Delaware, USA). Emerged flies and parasitoids were killed in absolute ethanol and stored at − 20 °C for subsequent species identification. Part of the emerged parasitoids were used to establish quarantine colonies at Berkeley laboratory. All puparia were later dissected under a binocular microscope (60×) and sorted as: puparia bearing either a fly or parasitoid emergence hole, unhatched puparia bearing a dead fly, unhatched puparia bearing a dead parasitoid (larva, pupa or adult), or unhatched puparia dead for unknown reasons. Percentage parasitism was calculated by dividing the sum of parasitized puparia (bearing a parasitoid emergence hole or containing a dead parasitoid) by the total number of puparia, excluding those categorized as dead for unknown reasons (see Daane et al. 2016). In 2015, berries of *Osyris wightiana* Wall ex Wight were also collected and emerged adult parasitoids were treated as described above (Table 1); however, fly puparia were not isolated and the parasitism level was not determined. Percentage parasitism between different host plant species was compared by G-test of independence (McDonald 2014).

Molecular analysis of *Ganaspis brasiliensis*

Molecular analyses focused exclusively on *G. brasiliensis*, which was the most abundant parasitoid species emerging from *D. suzukii*-like puparia. Because Nomano et al. (2017) separated *G. brasiliensis* into five lineages, we sequenced the *cytochrome oxidase subunit I* (COI) gene to clarify our Chinese specimens' genetic identity and extend the knowledge on host range and geographic distribution of different *G. brasiliensis* lineages. Whole specimens were subjected to genomic DNA extraction using a nondestructive (without grinding the specimen) Chelex-proteinase K protocol (Gebiola et al. 2009). The COI gene of 30 individuals (Table 2) was amplified using the primer combination LCO and HCO (Folmer et al. 1994). Reactions were performed in 20 µl volumes containing 4 µl of 1X GoTaq buffer (Promega Corp., Madison, Wisconsin, USA), 1.6 µl dNTP (2.5 mM each), 1 µl of forward and reverse primer (10 µM each), 0.4 µl GoTaq G2 DNA Polymerase (Promega) (5u/µl), and 2 µl template DNA. Amplifications were achieved using a Bio-Rad thermocycler Mycycler (Bio-Rad, Hercules, California, USA) programmed at 1 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 90 s at 48 °C, and 60 s at 72 °C, and a final step of 7 min at 72 °C. PCR products were visualized after electrophoresis on 1% agarose gel stained with Gel Red™ (Biotium Inc, Fremont, California, USA) to confirm the

Table 2 *Ganaspis brasiliensis* specimens reared from *D. suzukii*-like puparia and used for COI gene sequencing

Host fruit	Location[a]	No. of specimen	Voucher code	GenBank accession No
Sambucus adnata	11	16	DSZ113-114, DSZ116-120, DSZ 122, DSZ129-131, DSZ133-137	MG755073-MG755088
Fragaria moupinensis	11	2	DSZ141, DSZ 144	MG755089, MG755091
Osyris wightiana	4	1	DSZ142	MG755090,
Rubus foliosus	10	8	DSZ097, DSZ147, DSZ186-191	MG755072, MG755092-MG755098
Rubus niveus	10	3	DSZ197-199	MG755099-MG755101

[a] See Table 1

amplification. Fragments obtained were sequenced in both sense and antisense directions by adopting EZ-seq standard service (Macrogen Inc., Seoul, South Korea). The chromatograms obtained were viewed and edited in Chromas v.2.6.4 (Technelysium, South Brisbane, Queensland, Australia). COI sequences of *G. brasiliensis* were deposited in GenBank under the accession numbers MG755073-MG755101 (Table 2), and parasitoid wasps were vouchered at the CNR, Institute for Sustainable Plant Protection, Unit of Portici, Italy. COI sequences were aligned using the Muscle alignment tool in Aliview 1.18.1 (Lasson 2014). Protein coding was checked by translating the sequences into amino acids. No evidence for the presence of pseudogenes (i.e., no stop codons or frame shifts) was detected.

Phylogenetic analysis of COI sequences was performed by Bayesian inference (BI) using MrBayes 3.2.6 (Ronquist et al. 2012). The BI tree was obtained by implementing a different substitution model for each codon position, that is GTR + I + G, GTR + I and GTR + G for codon 1, 2 and 3, respectively. Substitution models were selected by PartitionFinder version 2.1.1 (Lanfear et al. 2012), based on the AICc criterion (AIC for small sample size). BI was conducted according to the best partitioning scheme selected by PartitionFinder using ‘all’ search algorithm with branch lengths linked. Two runs of four Monte Carlo Markov chains (3 ‘heated’ and 1 ‘cold’) were run in parallel in MrBayes for 5,000,000 generations, and trees were sampled every 1000 generations. Convergence of the separate runs was checked using the average deviation of split frequencies diagnostic (< 0.01) and the PSRF parameter (close to 1.00 for all parameters). The burn-in value was set at 25% of sampled topologies. Trees were imported into the tree editor TreeGraph 2.14.0-771 beta (Stover and Muller 2010) for annotation and layout. COI sequences of *G. brasiliensis* from Nomano et al. (2017) were added to the ingroup. *Ganaspis xanthopoda* was used as an outgroup.

Quarantine test of host specificity of *G. brasiliensis*

A host specificity test using Yunnan-collected *G. brasiliensis* was conducted under controlled conditions (22 ± 2 °C, 14L: 10D, 40–60% RH) in a quarantine at the University of California, Berkeley. The *D. suzukii* larval colony was maintained in Petri dishes (1.5 × 8 cm) filled with a cornmeal-based artificial diet and that had been inoculated by exposure to adult flies for a 24-h period in organdy-screened cages (see Wang et al. 2018). Colonies of native non-target species tested were initiated from specimens purchased from the University of California’s *Drosophila* Stock Center in San Diego, California, where these species were originally collected from different locations in the USA. Nine non-target species were tested: *Drosophila simulans* Sturtevant, *D. melanogaster* Meigen, *D. persimilis* Dobzhansky and Epling, *D. pseudoobscura* Frolova, *D. busckii* Coquillett, *D. montana* Stone, Griffen & Patterson, *D. robusta* Sturtevant, *D. funebris* (Fabricius) and *Hirtodrosophila duncani* Sturtevant (Table S1). Non-target species selection was based on their phylogenetical relationship to *D. suzukii*: *D. simulans* and *D. melanogaster* are closely related to *D. suzukii* (all belong to the *D. melanogaster* species group), whereas the other species are more distantly related (Markow and O’Grady 2006). The colony of *G. brasiliensis* was initiated from the 2016 collections in Yunnan, China, by combining the progeny of 45 female parasitoids identified morphologically as *G. brasiliensis* and collected at either Chang Chong Shan (Wu Hua District) or Dong Da Cun (Pan Long District) (Table 1). At that time, we had no information on the genetic variability of *G. brasiliensis* (Nomano et al. 2017), and for this reason, we did not genetically characterize the colony. Subsequent molecular characterization of the original field populations of *G. brasiliensis* revealed that the colony originated from a mixture of G1 and G3 lineages (lineages described by Nomano et al. 2017) (see below, results of molecular analysis of *G. brasiliensis*). Since the collection in China, the colony was maintained on *D. suzukii* larvae for 3–5 generations. Previously, we found that *G. brasiliensis* prefers to attack young larvae (Wang et al. 2018) and, for this reason, we used 1–2-day-old *D. suzukii* larvae for the established colony. For the rearing of this parasitoid, 40 larvae were transferred from the Petri dish colony to a small plastic vial (25 × 95 mm) filled with diet and immediately exposed to two mated, 3–4-day-old female *G. brasiliensis* for a 2-day period. Vials of exposed larvae were then held for the emergence of adult flies or parasitoids, with emerged adult parasitoids transferred to plastic vials supplied with 50% honey water streaked on the vial plug; the adults were later used for colony maintenance or quarantine trials.

The non-target test consisted of no-choice exposures to determine whether *G. brasiliensis* could attack and develop from the drosophilid species tested. For each replicate, 20 host larvae were carefully transferred under a stereomicroscope using a soft and fine brush from the Petri dish colonies to a plastic vial (25 × 95 mm) filled with 1 cm cornmeal diet and then exposed to a single mated, 3–4-day-old naïve female wasp for a 24-h period. Our preliminary observations showed negligible mortality caused by the transfer. Exposed fly larvae were held in these vials until the emergence of flies and wasps. There were 24 or 25 replicates for each fly species, except for *H. duncani* (20 replicates due to the shortage of host larvae) and *D. robusta* (30 replicates) (i.e., 20–30 female wasps tested for each fly species). The tests were conducted in five consecutive days, and there were five positive control replicates (each consisting of 20 *D. suzukii* larvae similarly exposed to *G. brasiliensis*) for each testing date and totally five negative control replicates (unexposed fly larvae held under the same conditions) for each tested fly species. After insect emergence ceased, all dead pupae were reconstituted in water for 1 day and then dissected under a microscope to determine the presence or absence of recognizable fly or parasitoid cadavers (e.g., pharate adults).

The 'Success rate of Parasitism' (SP), which measures the probability that a parasitized host will give rise to an adult wasp, was estimated as $p_i/(T-d_i)$, where p_i = the number of emerged parasitoids, T = the number of emerging flies in the absence of the parasitoid, and d_i = the number of emerged flies in the presence of the parasitoid (Chabert et al. 2012). If $p_i > (T-d_i)$, we set SP = 1 (Chabert et al. 2012). We included developed but dead flies and wasps from the dissection of dead pupae to estimate the total number of emerged flies or wasps for a more precise estimate of SP (Kaçar et al. 2017). To examine possible encapsulation of the parasitoid eggs or larvae by the hosts, all emerged flies were checked for the presence of black capsules inside the fly's abdomen (Chabert et al. 2012; Wang et al. 2016b). All positive controls (totally 25 replicates) for *D. suzukii* were pooled. The number of emerged wasps and SP were compared among the 10 different drosophilid species using one-way ANOVA. Prior to the analyses, all percentage data were arcsine, square root transformed to normalize the variance after checking the normality of residuals and homoscedasticity with Shapiro's and Bartlett's test. We also compared the numbers of developed adult flies in the presence (treatment) and absence (negative control) of parasitoids. Mean values among different treatments were separated using Tukey's HSD. All analyses were conducted using JMP® Pro 13 (SAS, Cary, NC).

Results

Parasitoids from fruit-baited traps

A total of 458 adult parasitoids of drosophilid flies were collected from fruit-baited traps from 2013 to 2015, with majority being Braconidae (49.56%), followed by Figitidae (37.55%), Diapriidae (7.42%), and Pteromalidae (5.46%) (Table S2). Seven *Asobara* species were collected (Tables 3, S2–S5), among which *Asobara mesocauda* van Achterberg and Guerrieri (36.46%) and *A. brevicauda* (9.17%) were the most abundant. Only a few individuals of other *Asobara* species were collected. Four genera of Figitidae were collected; the majority of specimens reared belonged to *Leptopilina*, with *L. j. japonica* resulting the most abundant (14.41%), followed by *L. decemflagella* Lue & Buffington (5.46%). From these fruit-baited traps, *Ganaspis* species were the least represented figitids (0.22%) with only a single individual of *G. xanthopoda* collected (Tables 3, S2–S5); other figitids collected included two new species: one belonging to an undescribed genus related to *Leptopilina* (on the basis of our unpublished morphological and molecular analysis) (11.35%) and one to the genus *Leptolamina* (6.11%) (Tables 3, S2–S5). As for the new genus, 50 of 52 individuals collected were females, suggesting a possible thelytokous reproduction. The Diapriidae and Pteromalidae collected were *T. drosophilae* and *P. vindemiae*, respectively. Identification of a sub-sample of 1707 drosophilid flies emerged from the fruit-baited traps found only 18 *D. suzukii* (1.05%) and four *D. pulchrella* (0.23%) (Table S6).

Table 3 Composition of drosophila parasitoids collected by banana-baited traps during 2013–2015 in Yunnan Province, China

Family	Parasitoid species	No. of emerged wasps per year				Overall composition (%)
		2013	2014	2015	Total	
Braconidae	*Asobara brevicauda*	6	6	30	42	9.17
	A. elongata	3	0	0	3	0.65
	A. japonica	1	0	0	1	0.22
	A. leveri	1	0	0	1	0.22
	A. mesocauda	62	96	9	167	36.46
	A. unicolorata	12	0	0	12	2.62
	A. triangulata	1	0	0	1	0.22
Figitidae	*Leptopilina japonica japonica*	3	8	55	66	14.41
	L. decemflagella	5	7	13	25	5.46
	Leptolamina sp.	23	4	1	28	6.11
	New genus related to *Leptopilina*	18	3	31	52	11.35
	Ganaspis xanthopoda	1	0	0	1	0.22
Pteromalidae	*Pachycrepoideus vindemiae*	1	0	24	25	5.46
Diapriidae	*Trichopria drosophilae*	0	7	27	34	7.42

Parasitoids from soft berries

From fruit collections, 14,183 *D. suzukii*-like puparia were recovered during July 2016: 737 from *R. foliosus*, 4504 from *R. niveus*, 2456 from *F. moupinensis*, and 6486 from *S. adnata* (Tables 4, S7). Overall, 60.9% of these puparia were classified as dead for unknown reasons. This high mortality could have been caused by various factors, including impairment of puparia during collection, suboptimal environmental conditions during transport from China to USA quarantine laboratories, some dehydration, and host killing by parasitoids (ovipositor sting not followed by egg laying, although, having found a very low number of pupal parasitoids, this factor may have affected the mortality only marginally). As it was not possible to understand the cause of single puparia mortality and if parasitized or not, puparia dead for unknown reasons were excluded from calculation of percentage parasitism. Across all sample dates and sites, 48.9% of the remaining 5550 puparia were parasitized. Percentage parasitism (Table 4) did not vary substantially among puparia collected from berries of *R. foliosus* (22.35%), *R. niveus* (18.81%), and *F. moupinensis* (19.75%), whereas puparia collected from *S. adnata* had the highest percentage parasitism at 63.46% (*G*-test of independence, $G=1000.36$, $df=3$, $P<0.0001$). A similar result was obtained when the analysis was restricted to location 11 where most of the puparia were collected and three host plants (*R. foliosus*, *F. moupinensis,* and *S. adnata*) occurred simultaneously (G-test of independence, $G=735.24$, $df=2$, $P<0.0001$). Finally, percentage parasitism differed between location 10 (20.58%), where only *Rubus* berries were found, and location 11 (53.94%), characterized by the occurrence of berries of different botanical origin (G-test of independence, $G=300.19$, $df=2$, $P<0.0001$).

The majority of collected adult parasitoids were figitids (*G. brasiliensis* at 65.4%, *L. j. japonica* at 32.9%), with braconids (*Asobara* spp. at 0.6%) and diapriids (*T. drosophilae* at 0.6%) occurring in only a few samples (Table S8). Three different *Asobara* species were found: *A. mesocauda* from *R. niveus* and *F. moupinensis*, *A. leveri* from *R. niveus*, and *A. unicolorata* van Achterberg and Guerrieri from *S. adnata*. *Trichopria drosophilae* emerged only from puparia collected from *S. adnata*. Host fruit species influenced the ratio between *G. brasiliensis* and *L. j. japonica* (Fig. 1), with *G. brasiliensis* more abundant than *L. j. japonica* on *R. foliosus* (80% vs 20%), *F. moupinensis* (96.8% vs 2.7%) and *S. adnata* (64.7% vs 34.4%), whereas *L. j. japonica* was dominant over *G. brasiliensis* on *R. niveus* (71.4% vs. 25.7%) (G-test of independence, $G=204.75$, $df=3$, $P<0.0001$) (Fig. 1, Table S8). *Ganaspis brasiliensis* was the most abundant species collected, reaching a parasitism rate of 31.54% on average, ranging from 4.31% (*R. niveus*) to 40.42% (*S. adnata*). Parasitism rate by *L. j. japonica* was 16.18% on average, ranging from 4.47% (*R. foliosus*) to 21.52% (*S. adnata*) (Fig. 1). As for *O. wightiana* berries (collected in 2015), only two *G. brasiliensis* and one *L. decemflagella* emerged from infested fruits.

Taxonomic identification of flies emerging from unparasitized puparia revealed the occurrence of both *D. suzukii* and *D. pulchrella*. *Drosophila suzukii* was the less frequent of the two (on average 12.4%) (Table S9), with no significant difference among host fruits (Fisher's exact Test of independence, $df=3$, $P=0.154$).

Table 4 Numbers of fruits and hosts (*Drosophila suzukii* and *D. pulchrella*) puparia collected and percentage parasitism on different host fruits from different locations in 2016 in Yunnan Province, China

Host fruit	Location[a]	No. of fruits	No. of hosts puparia collected				% Parasitism[e]			
			Total	Dead[b]	Unparasitized[c]	Parasitized[d]	Figitidae	Braconidae	Diapriidae	Total
Rubus foliosus	10	1900	500	431	72	25	36.23	0.0	0.0	36.23
	11	355	237	136	153	13	19.40	0.0	0.0	19.40
	All	2255	737	567	225	38	22.35	0.0	0.0	22.35
Rubus niveus	9	477	553	534	32	3	10.53	5.26	0.0	15.79
	10	3404	3951	3263	1085	130	18.13	0.73	0.15	19.01
	All	3881	4504	3797	1117	133	16.17	2.49	0.15	18.81
Fragaria moupinensis	11	5951	2456	1489	1545	191	19.54	0.21	0.0	19.75
Sambucus adnata	11	70,177	6486	2780	2177	2352	61.94	0.95	0.57	63.46
All samples			14,183	8633	5063	2714	47.72	0.82	0.36	48.90

[a] See Table 1

[b] Puparia dead for unknown reasons

[c] Puparia with adult flies emerged or dead flies inside

[d] Puparia with adult parasitoids emerged or dead parasitoid larvae or pupae inside

[e] Parasitism = parasitized puparia × 100/(total puparia − dead puparia)

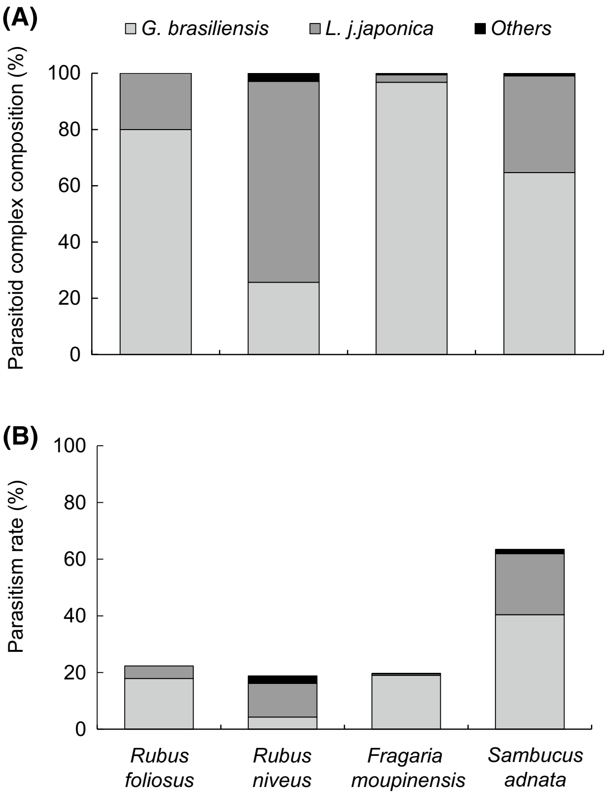


Fig. 1 **a** Composition of the parasitoid complex and **b** parasitism rate of hosts (*Drosophila suzukii* and *D. pulchrella*) on four different host fruits. Others include the parasitoids *Asobara mesocauda, A. unicolorata, A. leveri,* and *Trichopria drosophilae*

Molecular analysis of *G. brasiliensis*

Trimmed COI sequences of *G. brasiliensis* showed a fragment size of 635 nucleotides and 68 polymorphic sites. Alignment was straightforward with no frame shifts, nonsense codons, insertions or deletions identified in any sequence. BI analysis produced a tree (Fig. 2) showing that *G. brasiliensis* from Yunnan clustered in two groups corresponding to the G1 lineage (specialist on *D. suzukii*) and the G3 lineage (generalist on drosophilids), as described by Nomano et al. (2017). Of the 30 individuals analyzed, 27 (77%) were in the G1 and 7 (23%) in the G3 group. The G1–G5 lineages were highly supported (posterior probabilities 0.98, 1, 0.99, 1, and 0.93 for G1, G2, G3, G4, and G5 lineages, respectively).

Host specificity of *G. brasiliensis*

Ganaspis brasiliensis successfully developed from *D. suzukii, D. simulans*, and *D. melanogaster* and the number of offspring that developed and the SP were not significantly different among these three species (Fig. 3). Host species affected the number of offspring developed ($F_{9,236} = 18.6$, $P < 0.001$) and SP ($F_{9,236} = 27.9$, $P < 0.001$) (Fig. 3). Additionally, a few adult parasitoids developed from *D. montana* or *D. persimilis*, but there was no significant difference in the number of adult flies developed in the presence and absence of the parasitoid among all these seven non-target species (Fig. S1). In total, only three out of 292 *D. simulans* adults and one out of 323 *D. suzukii* adults contained black capsules, while all emerged flies of other species (totally 2370) did not contain them.

Discussion

Our surveys in Yunnan, China, discovered 15 parasitoid species reared from drosophilids in fruit-baited traps, but only six parasitoid species from field-collected fruits. This difference is also reflected in the species composition of the parasitoid complex, in the species prevalence and host–parasitoid associations. *Asobara* species dominated (49.56%) the parasitoid complex from the fruit-baited traps, whereas *G. brasiliensis* was dominant in field-collected fruits (65.4%). *Leptopilina j. japonica* was present in both sampling methods (14.41% in fruit-baited traps and 32.9% in fruit sampling). The most apparent difference between these sampling methods is that *D. suzukii* and *D. pulchrella* were the only drosophilid species emerging from fresh fruits, whereas they were largely absent from fruit-baited traps. This suggests that the fruit-bait attracted more diverse drosophilid species and consequently a higher diversity of parasitoids, but direct sampling of ripening and fresh fruits collected parasitoids specifically associated with *D. suzukii* and its close relative *D. pulchrella*. Similarly to our findings, a contemporary survey of ours carried out in China and Japan during 2015–2017 (Girod et al. 2018a) discovered eight larval parasitoid species emerging form fresh fruits infested by larvae of *D. suzukii* and the closely related species *D. pulchrella* and *D. subpulchrella*; among the collected larval parasitoids, *G. brasiliensis* was the most abundant, followed by *L. japonica*. It should be noted that Girod et al. (2018a) referred to *G. brasiliensis* as '*Ganaspis* cf. *brasiliensis*'; specimens from the project presented here were compared to those of Girod et al. (2018a), and indeed, all specimens conferred to *G. brasiliensis.*

Among the *Asobara* species, *A. japonica* was rarely collected in Yunnan, China, whereas it was the most common generalist parasitoid of drosophilids collected from fruit-baited traps in Japan and South Korea (Daane et al. 2016; Ideo et al. 2008; Mitsui and Kimura 2010; Mitsui et al. 2007). Our findings represent the first report of *A. japonica* from China. Among the species, we collected by fresh fruits infested by *D. suzukii* or *D. pulchrella*, *Asobara mesocauda* and *A. unicolorata* are currently known only from Yunnan Province (Guerrieri et al. 2016);

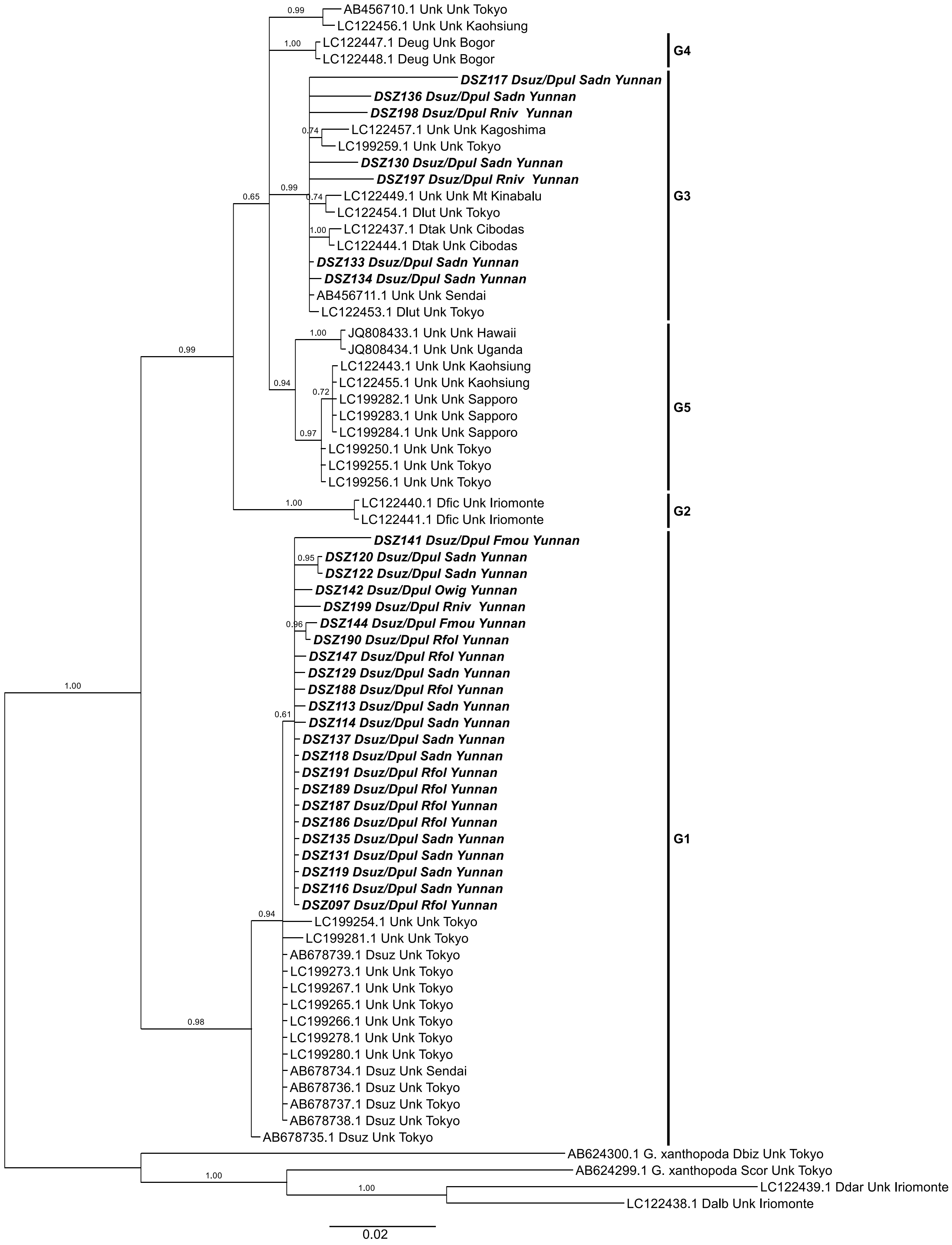
A. leveri was originally described from the Fiji Islands (Nixon 1939) and had previously been recorded from China (Hubei Province), Japan and South Korea (Guerrieri et al. 2016; Nomano et al. 2015) and was associated with *D. suzukii* in South Korea (Daane et al. 2016). *Asobara mesocauda*, *Asobara pleuralis* (Ashmead), and *Asobara* sp. TK1 (maybe *A. triangulata* sensu Guerrieri et al. 2016) were also isolated by infested fresh fruits by Girod et al. (2018a). In our collections, *A. brevicauda* was only recovered from fruit-baited traps, although it was collected from *D. suzukii* in South Korea (Daane et al. 2016). The large presence of *Asobara* species in the fruit-baited traps and its paucity in fruit collections suggests a greater propensity of these braconid parasitoids to attack host larvae developing in decaying fruits rather than fresh ones as is the case of *D. suzukii* and *D. pulchrella*.

Among the figitids, *L. decemflagella* represents the first report from China. This species was only recently described from Eastern North America, but its biology is so far unknown (Lue et al. 2016). *Leptopilina decemflagella* was frequently collected in fruit-baited traps, but it was never recovered from puparia collected from ripe fruits, suggesting that it is not likely associated with *D. suzukii* or *D. pulchrella*. Two new species records of figitids were also collected exclusively in fruit-baited traps and include *Leptolamina* sp. and a species of an undescribed genus related to *Leptopilina*. The large number of *L. j. japonica* collected with fruit-baited traps probably emerged from drosophilid species other than *D. suzukii* and *D. pulchrella*. In contrast, *G. brasiliensis* was isolated exclusively from *D. suzukii*-like puparia from field-collected fruits. This suggests that *L. j. japonica* could be a more generalist parasitoid than *G. brasiliensis*. Indeed, *L. j. japonica* can form new associations with some *Drosophila* species without undergoing adaptive changes (Kimura and Novkovic 2015). In fruit samples, *G. brasiliensis* co-occurred with *L. j. japonica* but was much more abundant than *L. j. japonica* on *R. foliosus*, *F. moupinensis* and *S. adnata* (the opposite was observed on *R. niveus*). Records of *G. brasiliensis* and *L. japonica* attacking larvae of *D. suzukii*, *D. pulchrella* or *D. subpulchrella* in fresh fruits have recently been reported from different provinces of China, and in particular from the Yunnan province (Girod et al. 2018a). One population of *L. japonica* from Yunnan was able to parasitize other two *Drosophila* species, including *D. melanogaster* and *D. subobscura* (the former is closely related and the latter more distantly related to *D. suzukii*) (Girod et al. 2018b).

The two pupal parasitoids, *T. drosophilae* and *P. vindemiae*, were collected from both fruit-baited traps and field-collected fruits, although in low numbers, in part because of the relatively short exposure period of fruit-baited traps and host larvae that had already left the fruits collected in the field.

Our results also indicate an effect of the host plant species on parasitism rate and parasitoid species composition. Overall, we found a considerable rate of parasitism of fly larvae developing in fresh fruits, ranging from 19 to 22% on *R. foliosus*, *R. niveus,* and *F. moupinensis* to 63% on *S. adnata*. The parasitism activity was almost entirely due to *G. brasiliensis* and *L. j. japonica*, with the former being the most active. On *R. foliosus* and *F. moupinensis*, 18–19% of puparia were parasitized by *G. brasiliensis*, whose parasitism rate reached 40% on *S. adnata*. *Leptopilina j. japonica* was more active than *G. brasiliensis* only on *R. niveus* (12% vs 4% parasitized puparia). The concentrated sampling period (July) may have affected the parasitism rate and the proportion of the host species due to the climate and fruit availability. However, our results are in line with those reported by Girod et al. (2018a) who found the total parasitism rate ranging from 0–54% in China and 0–76% in Japan, depending on the host plant and the time of collection, with *G. brasiliensis* being the most active larval parasitoid, reaching the maximum parasitism rate of 42% on *Prunus cerasoides* in Yunnan and 76% on *Morus* sp. in Japan.

Nomano et al. (2017) recognized five lineages of *G. brasiliensis*, which differed in geographic distribution and host range: G1, including individuals from Japan parasitizing only *D. suzukii*; G2, including individuals from a subtropical Japanese isle parasitizing *Drosophila ficusphila* Kikkawa & Peng; G3, including individuals from temperate regions of Japan and high mountains of Southeast Asia (Indonesia, Malaysia) parasitizing different species of *Drosophila*, except *D. suzukii*; G4, including individuals from Indonesia parasitizing *Drosophila eugracilis* Bock and Wheeler; G5, including individuals from Japan, Taiwan, Hawaii, and Uganda, from unknown host(s). In the laboratory, individuals belonging to G5 from Hawaii and Uganda have shown a poor ability to parasitize *D. suzukii* (Kacsoh and Schlenke 2012). Phylogenetic analysis of COI sequences revealed that our *G. brasiliensis* samples were grouped in two lineages: 77% in the G1 lineage and the remaining 23% in the G3 lineage. Their occurrence was sympatric on the same host plants, in accordance with previous findings indicating that G1, G3, and G5 are sympatric in Japan (Nomano et al. 2017). Although morphologically indistinguishable from each other, theses lineages could be a complex of cryptic species. Nomano et al. (2017) found incomplete reproductive isolation between populations of G3 and G5 lineages which group in the same clade. The level of reproductive isolation between the G1 lineage and the other lineages has not been investigated yet. However, the genetic distance (calculated on the COI gene nucleotide sequence) between the G1 individuals and those of other lineages is large enough (5–7% between G1 and G3 individuals) to suggest a reproductive isolation. Also, the higher level of host specificity of G1 lineage in respect to more generalist ones (Nomano


0.99
AB456710.1 Unk Unk Tokyo
LC122456.1 Unk Unk Kaohsiung
1.00
LC122447.1 Deug Unk Bogor
LC122448.1 Deug Unk Bogor
G4
DSZ117 Dsuz/Dpul Sadn Yunnan
DSZ136 Dsuz/Dpul Sadn Yunnan
DSZ198 Dsuz/Dpul Rniv Yunnan
0.74
LC122457.1 Unk Unk Kagoshima
LC199259.1 Unk Unk Tokyo
DSZ130 Dsuz/Dpul Sadn Yunnan
DSZ197 Dsuz/Dpul Rniv Yunnan
0.65
0.99
0.74
LC122449.1 Unk Unk Mt Kinabalu
LC122454.1 Dlut Unk Tokyo
1.00
LC122437.1 Dtak Unk Cibodas
LC122444.1 Dtak Unk Cibodas
DSZ133 Dsuz/Dpul Sadn Yunnan
DSZ134 Dsuz/Dpul Sadn Yunnan
AB456711.1 Unk Unk Sendai
LC122453.1 Dlut Unk Tokyo
G3
1.00
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JQ808434.1 Unk Unk Uganda
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0.94
0.72
LC122443.1 Unk Unk Kaohsiung
LC122455.1 Unk Unk Kaohsiung
LC199282.1 Unk Unk Sapporo
LC199283.1 Unk Unk Sapporo
LC199284.1 Unk Unk Sapporo
0.97
LC199250.1 Unk Unk Tokyo
LC199255.1 Unk Unk Tokyo
LC199256.1 Unk Unk Tokyo
G5
1.00
LC122440.1 Dfic Unk Iriomonte
LC122441.1 Dfic Unk Iriomonte
G2
DSZ141 Dsuz/Dpul Fmou Yunnan
0.95
DSZ120 Dsuz/Dpul Sadn Yunnan
DSZ122 Dsuz/Dpul Sadn Yunnan
DSZ142 Dsuz/Dpul Owig Yunnan
DSZ199 Dsuz/Dpul Rniv Yunnan
0.96
DSZ144 Dsuz/Dpul Fmou Yunnan
DSZ190 Dsuz/Dpul Rfol Yunnan
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DSZ116 Dsuz/Dpul Sadn Yunnan
DSZ097 Dsuz/Dpul Rfol Yunnan
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LC199281.1 Unk Unk Tokyo
AB678739.1 Dsuz Unk Tokyo
LC199273.1 Unk Unk Tokyo
LC199267.1 Unk Unk Tokyo
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0.98
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LC199278.1 Unk Unk Tokyo
LC199280.1 Unk Unk Tokyo
AB678734.1 Dsuz Unk Sendai
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AB624300.1 G. xanthopoda Dbiz Unk Tokyo
1.00
AB624299.1 G. xanthopoda Scor Unk Tokyo
1.00
LC122439.1 Ddar Unk Iriomonte
LC122438.1 Dalb Unk Iriomonte
0.02

◂**Fig. 2** Bayesian phylogenetic tree for COI gene sequences of *Ganaspis brasiliensis*. Individuals sequenced in this work are reported in bold and italicized (see Table 2 for details; individuals retrieved from the NCBI database and occurring in Nomano et al. (2017) are identified by the GenBank accession number). Acronyms indicate the drosophilid host and fruit host from which each individual sample originated. *Drosophila*: Dalb (*D. albomicans*), Dbiz (*D. bizonata*), Ddar (*D. daruma*), Deug (*D. eugracilis*), Dfic (*D. ficusphila*), Dlut (*D. lutescens*), Dsuz (*D. suzukii*), Dsuz/pul (*D. suzukii* and *D. pulchrella*), Scor (*Scaptodrosophila coracina*) and unk (unknown). Host fruits: Fmou (*Fragaria moupinensis*), Owig (*Osyris wightiana*), Sadn (*Sambucus adnata*), Rfol (*Rubus foliosus*) and Rniv (*Rubus niveus*). Geographic origin of each individual sample is also reported. G1–G5 indicate *G. brasiliensis* lineages following Nomano et al. (2017). Posterior probabilities ≥ 0.60 are shown above the branches. *Ganaspis xanthopoda* AB624300 was used as outgroup. Scale bar indicates 0.02 changes per nucleotide position

et al. 2017; Matsuura et al. 2018; our results presented here) points toward the existence of a complex of species under the name *G. brasiliensis*. This hypothesis is also supported by the wide geographic distribution of this taxonomic entity (Asia, Central and South America) (Buffington and Forshage 2016). Studies are underway to disentangle the taxonomy of this putative complex of species.

In quarantine experiments, the studied population of *G. brasiliensis* from Yunnan, China (comprising G1 and G3 individuals), attempted to attack all tested fly species in artificial diet, which were provided in a simple no-choice test. These tests were conducted in small vial with artificial diet. The results thus reflect largely the suitability of the tested host larvae for the parasitoid's development (i.e., physiological host range), and the ecological host range could be further narrow. Indeed, quarantine experiments showed that *G. brasiliensis* successfully developed from *D. suzukii* and its two closed related hosts (*D. simulans* and *D. melanogaster*) but largely failed to develop from other seven tested host species. Nevertheless, rare encapsulated parasitoid eggs were noticed in emerged flies. Host species that are phylogenetically related may be attacked by common parasitoid species as they often share physiological or morphological characteristics that determine their suitability as a host (Desneux et al. 2012). Although both *D. melanogaster* and *D. simulans* are physiologically suitable hosts for *G. brasiliensis*, these drosophilids typically infest overripe or rotting fruits, while in the field *D. suzukii* exploits ripening fruit (Mitsui et al. 2007), before they are available to *D. melanogaster* or *D. simulans*. Indeed, our collections of fresh fruits rarely found drosophilid species other than *D. suzukii* and *D. pulchrella*. In addition, as a part of parasitoid host range drivers, host habitat (fresh vs rotten fruits) could be crucial for the host location success of drosophila larval parasitoids that mainly rely on specific host-derived volatile chemical cues (Biondi et al. 2017). In summary, we showed that *D. suzukii* and *D. pulchrella* co-occur on fresh fruit in Yunnan Province, where they are attacked by numerous parasitoid species, the most important of which is the more specialized *G. brasiliensis*, comprising the G1 and G3 lineages.

Geographic variations in the parasitoid virulence may also exist among host populations or lineages of *G. brasiliensis*. Our field observations and host specificity quarantine experiments suggest that both G1 and G3 lineages can successfully attack *D. suzukii*. Previously, the G1 lineage was considered a specialist on *D. suzukii* (Kasuya et al. 2013; Nomano et al. 2017); however, the characterization of different *G. brasiliensis* lineages in terms of host specialization may be premature based on a few samples from Japan. For example, Girod et al. (2018c) found that different *G. brasiliensis* geographic populations (two strains from Yunnan and one strain from Japan) successfully attacked *D. suzukii* developing in ripe blueberries, but rarely accepted fly larvae developing in artificial diet; two of these parasitoid populations (that were not characterized genetically) showed variations of the level of specificity, with the Japanese population being highly specific to *D. suzukii*, and a Yunnan population being able to parasitize, in addition to *D. suzukii*, larvae (in artificial diet) of *D. melanogaster* and rarely *D. subobscura* (Girod et al. 2018b). Here, we found similar level of host specificity in the tested population of *G. brasiliensis* from Yunnan. Interestingly, even in South Korea, we collected *G. brasiliensis* only from field-collected fruits infested by *D. suzukii* but not from traps baited with uninfested fruit (which yielded predominately other *Drosophila* species); this parasitoid population was able to develop successfully in larvae of *D. suzukii* and *D. melanogaster* in artificial diet (Daane et al. 2016). In another example, Mitsui and Kimura (2010) reported that a strain of *Ganaspis* from *Drosophila lutescens* Okada, which was later assigned to the G3 lineage by Nomano et al. (2017), parasitized only drosophilid larvae in fermenting fruits while *D. suzukii* in ripe fruits were rarely accepted. Our results extend to G3 lineage individuals the ability to parasitize both *D. suzukii* and *D. pulchrella*. More recently, Matsuura et al. (2018) suggested a level of specialization of one Japanese *D. suzukii*-associated type *G. brasiliensis* (G1 lineage) which attacked *D. suzukii* larvae in fresh fruits in the tree canopy and rarely larvae in fruits fallen on the ground. This result supports our findings of *G. brasiliensis* collected only from fresh fruit and never from fruit-baited traps. One explanation of this variation among populations of *G. brasiliensis* is that each of these lineages may have adaptated to local host species and host habitats (Murata et al. 2009; Rossi Stacconi et al. 2015). A different ability of G1 and G3 lineages of *G. brasiliensis* in parasitizing *D. suzukii* could be associated with a diverse capacity to overcome the strong cellular immune response of *D. suzukii* larvae (Kacsoh and Schlenke 2012; Poyet et al. 2013). Due to their high specificity toward *D. suzukii*, populations of *G. brasiliensis* represent good candidates for use in a classical biological control program. That some closely related

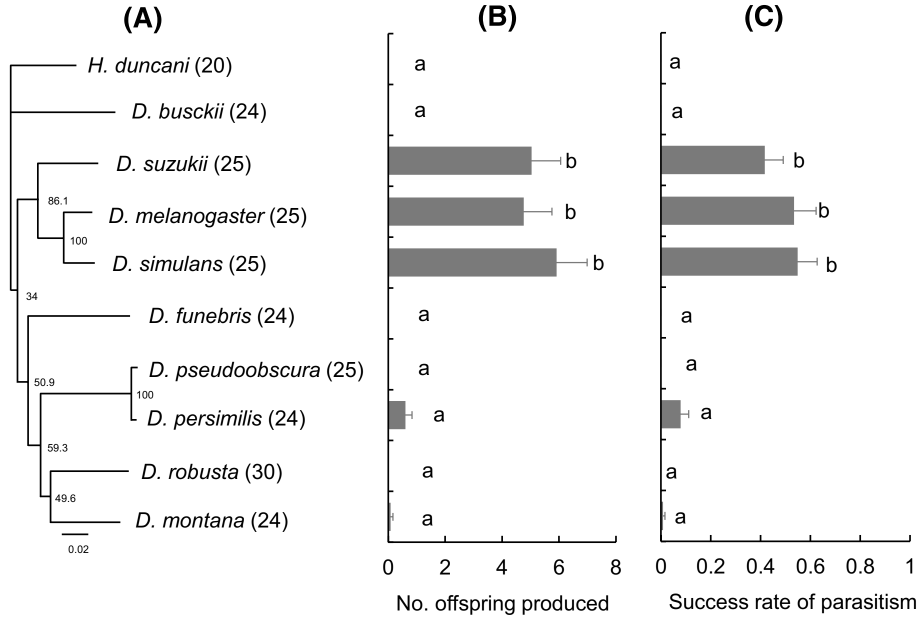

Fig. 3 **a** Phylogenetic tree of the host species tested in the quarantine experiment. The phylogenetic relationship was re-structured based on available COI gene sequences from NCBI database. **b** Number of offspring produced, and **c** success rate of parasitism by *Ganaspis brasiliensis* when tested with different *Drosophila* host species. Values are mean ± SE, and bars bearing different letters are significantly different (Tukey's HSD, $P < 0.05$). Number of replicates is reported in brackets after the name of each *Drosophila* species

species (i.e., *D. melanogaster* and *D. simulans*) might be attacked could also aid in biological control as these flies could serve as alternative hosts when *D. suzukii* are scarce. However, *G. brasiliensis* is unlikely to impact the populations of these non-target hosts because of competition with resident parasitoids that specialize on flies infesting rotted fruit. Detailed evaluations of the G1 and G3 lineages of *G. brasiliensis* are currently underway to determine their specific performance on *D. suzukii*, host range, and impact on non-target species with the goal of selecting a possible biological control agent to be introduced in the USA.

Author contribution

EG, MG, KMD, and KAH conceived and designed the project. MG, EG, KMD, and XGW wrote the initial manuscript; all co-authors helped editing the manuscript thereafter. EG, MB, MG, PC, GF, and GAC completed the molecular and taxonomic work; XGW, EH, AB, and KMD conducted the quarantine work. EG, MG, XGW, YW, FSC, HMZ, ZQC, HYC, CXL, KMD, and KAH conducted the field collections in China. All authors read, revised, and approved the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Asplen MK, Anfora G, Biondi A, Choi D-S et al (2015) Invasion biology of spotted wing drosophila (*Drosophila suzukii*): a global perspective and future priorities. J Pest Sci 88:469–494. https://doi.org/10.1007/s10340-015-0681-z

Beers EH, Van Steenwyk RA, Shearer PW, Coates WW, Grant JA (2011) Developing *Drosophila suzukii* management programs for sweet cherry in the western United States. Pest Manag Sci 67:1386–1395. https://doi.org/10.1002/ps.2279

Biondi A, Wang X, Miller JC, Miller B et al (2017) Innate olfactory responses of *Asobara japonica* toward fruits infested by the invasive spotted wing Drosophila. J Insect Behav 30:495–506. https://doi.org/10.1007/s10905-017-9636-y

Buffington ML, Forshage M (2016) Redescription of *Ganaspis brasiliensis* (Ihering, 1905), new combination (Hymenoptera: Figitidae), a natural enemy of the invasive *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae). Proc Entomol Soc Wash 118:1–13. https://doi.org/10.4289/0013-8797.118.1.1

Carton Y, Boulétreau B, van Alphen JJM, van Lenteren JC (1986) The *Drosophila* parasitic wasps. In: Ashburner M, Carson HL, Thompson JN (eds) The genetics and biology of *Drosophila*, vol 3e. Academic Press, London, pp 347–394

Chabert S, Allemand R, Poyet M, Eslin P, Gibert P (2012) Ability of European parasitoids (Hymenoptera) to control a new invasive Asiatic pest, *Drosophila suzukii*. Biol Control 63:40–47. https://doi.org/10.1016/j.biocontrol.2012.05.005

Daane KM, Wang X-G, Biondi A, Miller B et al (2016) First exploration of parasitoids of *Drosophila suzukii* in South Korea as potential classical biological agents. J Pest Sci 89:823–835. https://doi.org/10.1007/s10340-016-0740-0

Desneux N, Blahnik R, Delebecque CJ, Heimpel GE (2012) Host phylogeny and specialisation in parasitoids. Ecol Lett 15:453–460

Fleury F, Gibert P, Ris N, Allemand R (2009) Ecology and life history evolution of frugivorous *Drosophila* parasitoids. In: Prevost G (ed) Advances in parasitology: parasitoids of *Drosophila*. Advances in parasitology, vol 70. Academic Press, New York, pp 3–44. https://doi.org/10.1016/s0065-308x(09)70001-6

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Marine Biol Biotechnol 3:294–299

Gabarra R, Riudavets J, Rodríguez GA, Pujade-Villar J, Arnó J (2015) Prospects for the biological control of *Drosophila suzukii*. Biocontrol 60:331–339

Gebiola M, Bernardo U, Monti MM, Navone P, Viggiani G (2009) *Pnigalio agraules* (Walker) and *Pnigalio mediterraneus* Ferriere and Delucchi (Hymenoptera: Eulophidae): two closely related valid species. J Nat Hist 43:2465–2480. https://doi.org/10.1080/00222930903105088

Girod P, Borowiec N, Buffington M, Chen G et al (2018a) The parasitoid complex of *D. suzukii* and other fruit feeding *Drosophila* species in Asia. Sci Rep 8:11839. https://doi.org/10.1038/s41598-018-29555-8

Girod P, Lierhmann O, Urvois T, Turlings TCJ, Kenis M, Haye T (2018b) Host specifcity of Asian parasitoids for potential classical biological control of *Drosophila suzukii*. J Pest Sci 91:1241–1250. https://doi.org/10.1007/s10340-018-1003-z

Girod P, Rossignaud L, Haye T, Turlings TCJ, Kenis M (2018c) Development of Asian parasitoids in larvae of *Drosophila suzukii* feeding on blueberry and artificial diet. J Appl Entomol 142:483–494. https://doi.org/10.1111/jen.12496

Guerrieri E, Giorgini M, Cascone P, Carpenito S, van Achterberg C (2016) Species diversity in the parasitoid genus *Asobara* (Hymenoptera: Braconidae) from the native area of the fruit fly pest *Drosophila suzukii* (Diptera: Drosophilidae). PLoS ONE. https://doi.org/10.1371/journal.pone.0147382

Hauser M (2011) A historic account of the invasion of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. Pest Manag Sci 67:1352–1357. https://doi.org/10.1002/ps.2265

Haye T, Girod P, Cuthbertson AGS, Wang XG et al (2016) Current SWD IPM tactics and their practical implementation in fruit crops across different regions around the world. J Pest Sci 89:643–651. https://doi.org/10.1007/s10340-016-0737-8

Ideo S, Watada M, Mitsui H, Kimura MT (2008) Host range of *Asobara japonica* (Hym.: Braconidae), a larval parasitoid of drosophilid flies. Entomol Sci 11:1–6

Kaçar G, Wang XG, Biondi A, Daane KM (2017) Linear functional response by two pupal *Drosophila* parasitoids foraging within single or multiple patch environments. PLoS ONE 12(8):e0183525. https://doi.org/10.1371/journal.pone.0183525

Kacsoh BZ, Schlenke TA (2012) High hemocyte load is associated with increased resistance against parasitoids in *Drosophila suzukii*, a relative of *D. melanogaster*. PLoS ONE 7:e34721. https://doi.org/10.1371/journal.pone.0034721

Kasuya N, Mitsui H, Ideo S, Watada M, Kimura MT (2013) Ecological, morphological and molecular studies on *Ganaspis* individuals (Hymenoptera: Figitidae) attacking *Drosophila suzukii* (Diptera: Drosophilidae). Appl Entomol Zool 48:87–92. https://doi.org/10.1007/s13355-012-0156-0

Kenis M, Tonina L, Eschen R, van der Sluis B et al (2016) Non-crop plants used as hosts by *Drosophila suzukii* in Europe. J Pest Sci 89:735–748. https://doi.org/10.1007/s10340-016-0755-6

Kimura MT, Novković B (2015) Local adaptation and ecological fitting in host use of the *Drosophila* parasitoid *Leptopilina japonica*. Ecol Res 30:499–505. https://doi.org/10.1007/s11284-015-1244-8

Klick J, Lee JC, Hagler JR, Bruck DJ, Yang WQ (2016) Distribution and activity of *Drosophila suzukii* in cultivated raspberry and surrounding vegetation. J Appl Entomol 140:37–46. https://doi.org/10.1111/jen.12234

Knoll V, Ellenbroek T, Romeis J, Collatz J (2017) Seasonal and regional presence of hymenopteran parasitoids of *Drosophila* in Switzerland and their ability to parasitize the invasive *Drosophila suzukii*. Sci Rep 7:e40697. https://doi.org/10.1038/srep40697

Lanfear R, Calcott B, Ho SY, Guindon S (2012) Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol Biol Evol 29:1695–1701. https://doi.org/10.1093/molbev/mss020

Lasson A (2014) AliView: a fast and lightweight alignment viewer and editor for large data sets. Bioinformatics (Oxford) 30:3276–3278. https://doi.org/10.1093/bioinformatics/btu531

Lee JC, Dreves AJ, Cave AM, Kawai S et al (2015) Infestation of wild and ornamental noncrop fruits by *Drosophila suzukii* (Diptera: Drosophilidae). Ann Entomol Soc Am 108:117–129. https://doi.org/10.1093/aesa/sau014

Lue CH, Driskell AC, Leips J, Buffington ML (2016) Review of the genus *Leptopilina* (Hymenoptera, Cynipoidea, Figitidae, Eucoilinae) from the Eastern United States, including three newly described species. J Hymenopt Res 53:35–76. https://doi.org/10.3897/jhr.53.10369

Markow TA, O'Grady PM (2006) Drosophila: a guide to species identification and use (chapter 1). Academic Press, London, p 13

Matsuura A, Mitsui H, Kimura MT (2018) A preliminary study on distributions and oviposition sites of *Drosophila suzukii* (Diptera: Drosophilidae) and its parasitoids on wild cherry tree in Tokyo, central Japan. Appl Entomol Zool 53:47–53. https://doi.org/10.1007/s13355-017-0527-7

Mazzetto F, Marchetti E, Amiresmaeili N, Sacco D et al (2016) Drosophila parasitoids in northern Italy and their potential to attack the exotic pest *Drosophila suzukii*. J Pest Sci 89:837–850. https://doi.org/10.1007/s10340-016-0746-7

McDonald JH (2014) Handbook of biological statistics, 3rd edn. SparkyHouse Publishing, Baltimore

Miller B, Anfora G, Buffington M, Daane KM et al (2015) Seasonal occurrence of resident parasitoids associated with *Drosophila suzukii* in two small fruit production regions of Italy and the USA. Bull Insectol 68:255–263

Mitsui H, Kimura MT (2010) Distribution, abundance and host association of two parasitoid species attacking frugivorous drosophilid larvae in central Japan. Eur J Entomol 107:535–540

Mitsui H, Van Achterberg K, Nordlander G, Kimura MT (2007) Geographical distributions and host associations of larval parasitoids of frugivorous Drosophilidae in Japan. J Nat Hist 41:1731–1738. https://doi.org/10.1080/00222930701504797

Murata Y, Ideo S, Watada M, Mitsui H, Kimura MT (2009) Genetic and physiological variation among sexual and parthenogenetic populations of *Asobara japonica* (Hymenoptera: Braconidae), a larval parasitoid of drosophilid flies. Eur J Entomol 106:171–178

Nixon GEJ (1939) Notes on Alysiinae with descriptions of three new species (Hym., Braconidae). Proc R Entomol Soc Lond Ser B Taxon 8:61–67

Nomano FY, Mitsui H, Kimura MT (2015) Capacity of Japanese *Asobara* species (Hymenoptera; Braconidae) to parasitize a fruit pest

Drosophila suzukii (Diptera; Drosophilidae). J Appl Entomol 139:105–113. https://doi.org/10.1111/jen.12141

Nomano FY, Kasuya N, Matsuura A, Suwito A, Mitsui H, Buffington ML, Kimura MT (2017) Genetic differentiation of *Ganaspis brasiliensis* (Hymenoptera: Figitidae) from East and Southeast Asia. Appl Entomol Zool 52:429–437. https://doi.org/10.1007/s13355-017-0493-0

Poyet M, Havard S, Prévost G, Chabrerie O, Doury G, Gibert P, Eslin P (2013) Resistance of *Drosophila suzukii* to the larval parasitoids *Leptopilina heterotoma* and *Asobara japonica* is related to haemocyte load. Physiol Entomol 38:45–53. https://doi.org/10.1111/phen.12002

Ronquist F, Teslenko M, van der Mark P, Ayres DL et al (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542. https://doi.org/10.1093/sysbio/sys029

Rossi Stacconi MV, Grassi A, Dalton DT, Miller B et al (2013) First field records of *Pachycrepoideus vindemiae* as a parasitoid of *Drosophila suzukii* in European and Oregon small fruit production areas. Entomologia 1:1–15

Rossi Stacconi MV, Buffington M, Daane KM, Dalton DT et al (2015) Host stage preference, efficacy and fecundity of parasitoids attacking *Drosophila suzukii* in newly invaded areas. Biol Control 84:28–35. https://doi.org/10.1016/j.biocontrol.2015.02.003

Rossi Stacconi MV, Panel A, Baser N, Ioriatti C, Pantezzi T, Anfora G (2017) Comparative life history traits of indigenous Italian parasitoids of *Drosophila suzukii* and their effectiveness at different temperatures. Biol Control 112:20–27

Stover BC, Muller KF (2010) TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. BMC Bioinform. https://doi.org/10.1186/1471-2105-11-7

Takamori H, Watabe H-A, Fuyama Y, Zhang Y-P, Aotsuka T (2006) *Drosophila subpulchrella*, a new species of the *Drosophila suzukii* species subgroup from Japan and China (Diptera: Drosophilidae). Entomol Sci 9:121–128

Van Timmeren S, Isaacs R (2013) Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection programs. Crop Prot 54:126–133. https://doi.org/10.1016/j.cropro.2013.08.003

Wang XG, Kaçar G, Biondi A, Daane KM (2016a) Life-history and host preference of *Trichopria drosophilae*, a pupal parasitoid of spotted wing drosophila. Biocontrol 61:387–397. https://doi.org/10.1007/s10526-016-9720-9

Wang XG, Stewart TE, Biondi A, Chavez B, Ingels C, Caprile J, Grant J, Walton VM, Daane KM (2016b) Population dynamics and ecology of *Drosophila suzukii* in Central California. J Pest Sci 89:701–712. https://doi.org/10.1007/s10340-016-0747-6

Wang XG, Nance A, Jones JML, Hoelmer KA, Daane KM (2018) Aspects of the biology and developmental strategy of two Asian larval parasitoids evaluated for classical biological control of *Drosophila suzukii*. Biol Control 121:58–65. https://doi.org/10.1016/j.biocontrol.2018.02.010

Wiman NG et al (2016) *Drosophila suzukii* population response to environment and management strategies. J Pest Sci 89:653–665. https://doi.org/10.1007/s10340-016-0757-4

Zhao C, Li P, Xie D-S, Hu C-H, Xiong Y, He L-Y, Li W-H, Xiao C, Yang P-Y, Li Z-Y (2017) The seasonal abundance of *Drosophila suzukii* in orchards and seasonal variation in fruit damage caused by this pest. Chin J Appl Entomol 54:724–729. https://doi.org/10.7679/j.issn.2095-1353.2017.088

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