



# Potential host ranges of three Asian larval parasitoids of *Drosophila suzukii*

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## Abstract

*Asobara japonica* (Hymenoptera: Braconidae), *Ganaspis brasiliensis* and *Leptopilina japonica* (Hymenoptera: Figitidae) are Asian larval parasitoids of spotted wing drosophila, *Drosophila suzukii* (Diptera: Drosophilidae). This study evaluated these parasitoids' capacity to attack and develop from 24 non-target drosophilid species. Results showed that all three parasitoids were able to parasitize host larvae of multiple non-target species in artificial diet; *A. japonica* developed from 19 tested host species, regardless of the phylogenetic position of the host species, *L. japonica* developed from 11 tested species; and *G. brasiliensis* developed from only four of the exposed species. Success rate of parasitism (i.e., the probability that an adult wasp successfully emerged from a parasitized host) by the two figitid parasitoids was low in hosts other than the three species in the melanogaster group (*D. melanogaster*, *D. simulans*, and *D. suzukii*). The failure of the figitids to develop in most of the tested host species appears to correspond with more frequent encapsulation of the parasitoids by the hosts. The results indicate that *G. brasiliensis* is the most host specific to *D. suzukii*, *L. japonica* attacks mainly species in the melanogaster group and *A. japonica* is a generalist, at least physiologically. Overall, the developmental time of the parasitoids increased with the host's developmental time. The body size of female *A. japonica* (as a model species) was positively related to host size, and mature egg load of female wasps increased with female body size. We discuss the use of these parasitoids for classical biological control of *D. suzukii*.

**Keywords** *Asobara* · Biological control · *Ganaspis* · Host range · Invasive species · *Leptopilina*

## Key Message

- *Asobara japonica*, *Ganaspis brasiliensis* and *Leptopilina japonica* are parasitoids of *Drosophila suzukii*.

- We tested physiological abilities of these parasitoids on *D. suzukii* and 24 non-target drosophilid species.
- *Asobara japonica* showed a broad host range.
- *Leptopilina japonica* broadly attacked about half of the non-target species.
- *Ganaspis brasiliensis* parasitized *D. suzukii* and three closely related species.

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## Introduction

Classical biological control is a useful strategy for invasive pest species when effective natural enemies are lacking in the invaded range (Hajek et al. 2016; Van Driesche et al. 2010). Assessing a natural enemies' host range is a critical step in developing classical biological control programs (Hoddle et al. 2020), as insect parasitoid species may attack phylogenetically related hosts that share physiological traits or ecological niches (Desneux et al. 2012). Non-target tests of candidate agents have become increasingly important as

part of a shifting paradigm in classical biological control from potential benefits to risks of releasing selected natural enemies (Heimpel and Cock 2018). Natural enemies that exhibit host species plasticity are rarely purposely released, even though this may aid in their establishment, as would natural enemies that exhibit plasticity to environmental tolerances. Here, the host range of three solitary endoparasitoids, *Asobara japonica* Belokobylskij (Hym.: Braconidae), *Leptopilina japonica* Novković & Kimura and *Ganaspis brasiliensis* (Ihering) (both Hym.: Figitidae), were evaluated as a part of a North American classical biological control program for the invasive *Drosophila suzukii* Matsumura (Dip.: Drosophilidae) (Daane et al. 2016; Giorgini et al. 2019).

*Drosophila suzukii* is native to East Asia but has invaded and widely established in regions of the Americas, Europe and North Africa (Asplen et al. 2015; Boughdad et al. 2021; Ferronato et al. 2019). *Drosophila suzukii* infests ripening fruits and is able to develop on many soft-skinned and damaged fruit crops as well as wild host plants (Kenis et al. 2016; Lee et al. 2015; Poyet et al. 2015; Stewart et al. 2014). Multiple insecticide applications are often needed to prevent economic losses to crops (Farnsworth et al. 2017; Mermer et al. 2020; Van Timmeren and Isaacs 2013), increasing management costs. Moreover, crop systems are challenged by repeated reinvasions of adult flies from non-crop or untreated-crop habitats that provide refuge for *D. suzukii* populations throughout the year (Hennig and Mazzi 2018; Santoiemma et al. 2019; Tonina et al. 2018; Wang et al. 2019b). Improved suppression by natural enemies could reduce fly populations in reservoir habitats before adult flies move into crop habitats, thereby reducing pest pressure (Lee et al. 2019).

Numerous parasitoid species, mostly belonging to Braconidae (*Asobara*), Figitidae (*Ganaspis* and *Leptopilina*), Diapriidae (*Trichopria*) and Pteromalidae (*Pachycrepoides*), attack frugivorous drosophilids, with most species reported from flies in fallen and decaying fruits (Carton et al. 1986). In contrast, surveys of naturally occurring parasitoids attacking *D. suzukii* in North America or Europe have shown only low levels of parasitism, typically from pupal parasitoids (*Trichopria drosophilae* Perkins and *Pachycrepoides vindemiae* (Rondani)) and little or no larval parasitism (reviewed by Lee et al. 2019). Larval parasitoids commonly found attacking *D. melanogaster* Meigen and other drosophilids in rotting fruits (e.g., *Asobara tabida* Nees, *Leptopilina heterotoma* (Thomson), and *Leptopilina bouvardi* Barbotin) rarely develop from *D. suzukii* due to the fly's immune resistance (Kacsoh and Schlenke 2012). It is likely that improved natural regulation of *D. suzukii* could be achieved through the introduction of more host-specific parasitoids that are not as susceptible to the fly's immune response. Explorations for co-evolved parasitoids in South Korea, China and Japan discovered several larval parasitoids

of *D. suzukii* (Daane et al. 2016; Giorgini et al. 2019; Girod et al. 2018a). Among them, *A. japonica*, *G. brasiliensis* and *L. japonica* were the dominant parasitoids, with *G. brasiliensis* and *L. japonica* predominantly or exclusively reared from *D. suzukii* and reaching parasitism rates > 70%.

Basic biological traits and potential competitive interactions of these three parasitoids have been evaluated in quarantine facilities in Switzerland (Girod et al. 2018b, c) and California (Biondi et al. 2017, 2021; Hougardy et al. 2019; Wang et al. 2018, 2019a, 2020, 2021). Non-target impacts were first reported on five European drosophilid species for populations of *G. brasiliensis* from Kunming, China and Tokyo, Japan; populations of *L. japonica* from Kunming and Beijing, China; and a population of *A. japonica* from Tokyo, Japan (Girod et al. 2018b). Prior to this work, there was evidence from field collections that *A. japonica* is a generalist parasitoid of drosophilids (Ideo et al. 2008; Nomano et al. 2015), whereas *L. japonica* appears to be an oligophagous species, observed to parasitize *D. suzukii* and several other *Drosophila* species from the *melanogaster* group (Kimura and Suwito 2015; Mitsui and Kimura 2010; Novkovic et al. 2011). *Ganaspis brasiliensis*' host range is more difficult to describe as some populations appear to specialize on *D. suzukii* (Girod et al. 2018a, b; Matsuura et al. 2018), whereas other populations have broader host ranges (Buffington and Forshage 2016; Giorgini et al. 2019; Kasuya et al. 2013). The identification of *G. brasiliensis* populations or the existence of cryptic species will be discussed later and is the focus of several ongoing studies in Europe and North America. Reported herein is the non-target evaluation of these three larval parasitoids against 24 drosophilid species found in North America. Due to the phylogenetic and ecological diversity in the *Drosophila* species tested, we used no-choice assays to assess host acceptance—i.e., can the parasitoid locate the host—and host suitability—i.e., can the parasitoid attack and develop from the host. Any studies on parasitoid host searching and acceptance conducted under the constraints of the quarantine are necessarily simplistic compared with the natural environment; still, this work is critical to the selection of candidate parasitoids by understanding their possible non-target risks.

## Materials and methods

Studies were conducted under controlled conditions ( $23 \pm 2$  °C, 14L: 10D (and natural light), 40–60% RH) at the University of California's Quarantine Facility (Berkeley, CA, USA). Colonies of the target host (*D. suzukii*) and three larval parasitoid species (*A. japonica*, *G. brasiliensis*, and *L. japonica*) were maintained using methods similar to previously reported studies (Biondi et al. 2017; Wang et al. 2019a, 2020). Briefly, the *D. suzukii* colony was initiated from field

collections of infested cherries in Parlier, CA, USA. Adult flies were held in Bug Dorm cages (BioQuip Products Inc., Rancho Dominguez, CA, USA) with 10% honey-water solution provided as food. Fly larvae were reared on a standard cornmeal and yeast diet in Petri dishes (1.5 cm high, 14.0 cm diameter) that had been exposed to adult flies for 24 h. For colony maintenance and experiments, flies that had developed into 1–2 d old larvae were used as all three parasitoids prefer younger host larvae (Wang et al. 2018, 2021).

Parasitoid colonies were initiated from field collections of *D. suzukii* in South Korea on wild *Rubus* spp. in 2014; details of collection sites, locations and the parasitoid species composition and numbers collected are presented in Daane et al. (2016). Adult parasitoids were held in clean (no diet) drosophila vials supplied with a streak of 50% honey-water until use for either colony maintenance or experiments. *Drosophila suzukii* was used as the rearing host for 4–5 generations prior to all tests. For parasitoid rearing, about 20 *D. suzukii* larvae were transferred to drosophila vials filled with 2 cm of artificial diet and then exposed to one 3–6 d old female parasitoid for 2–3 d. The larvae were then held for the emergence of adult flies (unparasitized flies emerged in 4–7 d) or parasitoids (emerged after 17–23 d, depending on the species and gender). Emerged wasps were collected daily and held in clean drosophila vials provided with 50% honey-water. The two figitid species were kept with males from adult emergence and were assumed to be mated, whereas virgin female *A. japonica* were used as this species has a strongly female-biased sex ratio and the tested strain was primarily parthenogenetic (Daane et al. 2016). Details on some aspects of the biology of these parasitoids, reared on *D. suzukii*, have been presented previously (Biondi et al. 2021; Girod et al. 2018a, c; Hougardy et al. 2019; Wang et al. 2018, 2019a, 2021).

### Selection of non-target *Drosophila* species

There are over 2,000 described *Drosophila* species worldwide, consisting of two subfamilies Steganinae and Drosophilinae. Steganinae is a relatively small subfamily, whereas the Drosophilinae consists of many more genera, including *Drosophila* that has over 1500 described species. Most *Drosophila* species are non-pest insects, breeding in decaying substrates, and only a few, including *D. suzukii*, are considered invasive pests in North America. Moreover, only 13 *Drosophila* are listed as endangered species (US Fish and Wildlife Services) and these are picture-wing or pomace *Drosophila* in the Hawaiian Islands, and no *Drosophila* species are listed as threatened or endangered in the continental USA. For non-target studies, we selected 24 species from North America that represented diversity in their geographical origins, phylogenetic relationships (2 subfamilies, 7 genera, 9 subgenera, 22 species groups) and ecological niches

(fruits, mushrooms, wood, flowers, sap). Table S1 shows their taxonomic positions, rearing diet, origins and feeding habits. With the exception of *D. suzukii*, all fly species were purchased from the University of California's San Diego Drosophila Stock Center, and were originally collected from 11 different US states, except for a Japanese species (*Scaptomyza elmoi* Takada, selected as a close relative of the endangered Hawaiian Drosophilae) and a Samoan species (*Samoaia leonensis* Wheeler and Kambysellis). Fly species were reared for 3–8 generations in three different artificial mediums using similar methods as described above for *D. suzukii*. We note here that the earlier work by Girod et al. (2018b) selected five common European drosophilid species based on their phylogenetic relatedness and sympatry with *D. suzukii*; these five species were also used in our study. In addition, we focused on species found in North America, as required for the North American Plant Protection Organization (NAPPO) guidelines (NAPPO 2015). We thus included *S. elmoi* as a representative of Hawaiian drosophilids; more rare or endangered Hawaiian species were not tested because they are often difficult to obtain and/or rear, and importation to the UC Berkeley quarantine would have involved additional permits as they are non-native to North America.

The phylogenetic relationship of the tested drosophilid species was constructed based on available COI gene sequences from the NCBI database, with the exception of *S. elmoi* and *Gitona americana* that did not have available gene sequences. For these two species, a DNA extraction was performed using DNeasy Blood & Tissue Kit (Qiagen). The target COI gene was amplified using polymerase chain reaction (PCR), using primer pair LepF1-short/LepR1-short (LepF1-short: 5'-ATTCAACCAATCATAAAGATAT-3' and LepR1-short: 5'-TAAACTTCTGGATGTCCAAAAA-3'). For each sample, a 1.0 µL sample DNA was mixed with 5.0 µL 5X Green GoTaq Reaction Buffer (ProMega), 0.2 µM dNTP, 0.2 µM each primer, and 0.2 µL Taq DNA Polymerase (New England BioLabs) in a total reaction volume of 25 µL. The thermal profile used was 94 °C for 1 min, 5 cycles of 94 °C for 1.5 min, 45 °C for 1.5 min, and 72 °C for 1.5 min, 35 cycles of 94 °C for 1 min, 50 °C for 1.5 min, and 72 °C for 1 min, and a final extension of 72 °C for 5 min. Following target COI amplification, samples were treated with ExoSAP (0.5 µL Exonuclease I, 0.5 µL Shrimp Alkaline Phosphatase, 1.0 µL 10X Exonuclease Reaction Buffer, and 5.0 µL PCR product), and run at 37 °C for 15 min, then at 80 °C for 15 min. Samples were then sequenced using an ABI 3730xl DNA Sequencer (Thermo Fisher Scientific).

### Host acceptance and offspring survival

Test procedures for each host and parasitoid species combination were similar and were conducted to ascertain the innate or physiological potential of parasitoids

to parasitize and survive in each host species. No-choice tests were used because innate parasitism could be masked in choice assays by a preference for higher-ranked hosts and by relative differences in host quality. For each test, twenty 2–d-old fly larvae were transferred to vials with artificial diet using a wet and soft brush. The vials were then streaked with 50% honey-water as food for the adult wasps, and a female parasitoid was placed in each vial for a 24 h period. The exposed larvae were then held for the emergence of adult flies (4–7 d) or parasitoids (17–23 d), which were recorded daily. Larvae of some *Drosophila* species are able to defend themselves from parasitoid eggs placed inside their bodies by surrounding the egg with blood cells that eventually melanize and form a black capsule surrounding the egg, which results in the death of the immature parasitoid by asphyxiation and is visible in developed adult flies (Chabert et al. 2012; Wang et al. 2016). Therefore, we also examined all emerged flies for the presence of a black capsule inside the fly's abdomen. There were 30 replicates for each host species (e.g., 600 exposed hosts for each species), and 20–25 replicates of unexposed fly larvae that served as a control to estimate the natural mortality under experimental conditions. Control mortality varied from 11.1 to 47.2% among host species ( $F_{24,491} = 6.2$ ,  $P < 0.001$ ), with the three species in the *melanogaster* group (*D. melanogaster*, *D. simulans* Sturtevant, and *D. suzukii*) having the lowest natural mortality (Fig. S1).

### Effect of host species on the body size and mature egg load of female *Asobara japonica*

*Asobara japonica* developed from a wider variety of host species than the other two parasitoids (see results) and, for this reason, we used it as a model to measure the effect of host species size on the body size and potential fecundity (mature egg load) of female parasitoids. A subsample of 20–25 female wasps that emerged from each of 16 different host species were measured for their hind tibia and ovipositor lengths to the nearest 0.001 mm with an ocular micrometer under a microscope and dissected for their mature egg load when the females were 5–6 d old (i.e., when mature egg load would have been highest; Wang et al. 2018, 2021). Emerged female wasps were held in vials (8 cm high, 2 cm diameter) with a streak of 20% honey water and were killed in the freezer immediately before dissection. The length  $l$  and width  $w$  of 25 individual pupae of each host species were measured, and the volume ( $V$ ) of the prolate ellipsoid puparium was calculated as a proxy measurement of host body size using the formula  $V = 4/3\pi \times (l/2) \times (w/2)^2$  (Otto and Mackauer 1998).

### Data analysis

Results are presented as mean  $\pm$  SE. As described in Biondi et al. (2021) for assessing the impact of fly larvae in fruit versus artificial diet, the impact of host species on the development of parasitoid offspring was compared using “Successful Rate of Parasitism” (SP) to determine the probability that a parasitized host would give rise to an adult wasp. SP is calculated as  $ep / (ef - efp)$ , where  $ep$  = number of emerged parasitoids,  $ef$  = the average number of emerged flies in the absence of parasitoids (control), and  $efp$  = number of emerged flies in the presence of parasitoids. In instances when  $ep > (ef - efp)$ , we set  $SP = 1$ . The proportion of hosts that were successfully parasitized was calculated using the “Degree of Parasitism” (DP), which is similar to Abbott's or Schneider–Orelli formula used to correct treatment mortality and is calculated as  $(ef - efp) / ef$ . When  $ef < efp$ , we set  $ef - efp = 0$ . “Encapsulation rate” (ER) was calculated as  $ER = bc / (ef - efp + bc)$ , where  $bc$  = the number of emerged flies bearing black capsules. The effects of host and parasitoid species, and their interaction on DP, SP or ER were analyzed using a Generalized Linear Model (GLM) with a binomial distribution and a logit link function; effects on the number of emerged wasps were analyzed using GLM with a Poisson distribution and a logit identity link function.

To determine if parasitoid performance parameters were correlated with fly phylogeny, Moran's  $I$ , a statistical measure of spatial autocorrelation, was calculated using the phylosignal function in the phylosignal package in R version 4.0.2 (R Core Team 2020). The values for Moran's  $I$  range from  $-1$  to  $+1$ , with more positive values indicating that the tested trait is more similar between closely related taxa than expected by chance; it has the advantage of being insensitive to tree size and balance, and does not assume an evolutionary model (Münkemüller et al. 2012).

For host body size and development time, data were excluded from host species with  $< 5$  emerged parasitoids and the remaining data were analyzed using one-way ANOVA. We were able to analyze relationships between mean development time of *A. japonica* and *L. japonica* for multiple host species, and between adult female *A. japonica* offspring body size and mature egg load and host size using linear regression and one-way ANOVA. Prior to analyses, proportion data (control host mortality) were arcsine square-root transformed to homogenize variances and all data were checked for the normality of residuals and homoscedasticity using Shapiro's and Bartlett's tests, respectively. If a significant difference was detected, pairwise comparisons were performed using Tukey HSD tests. Analyses were performed using JMP®, Pro 14 (SAS Institute Inc., Cary, NC, 1989–2019).

### Results

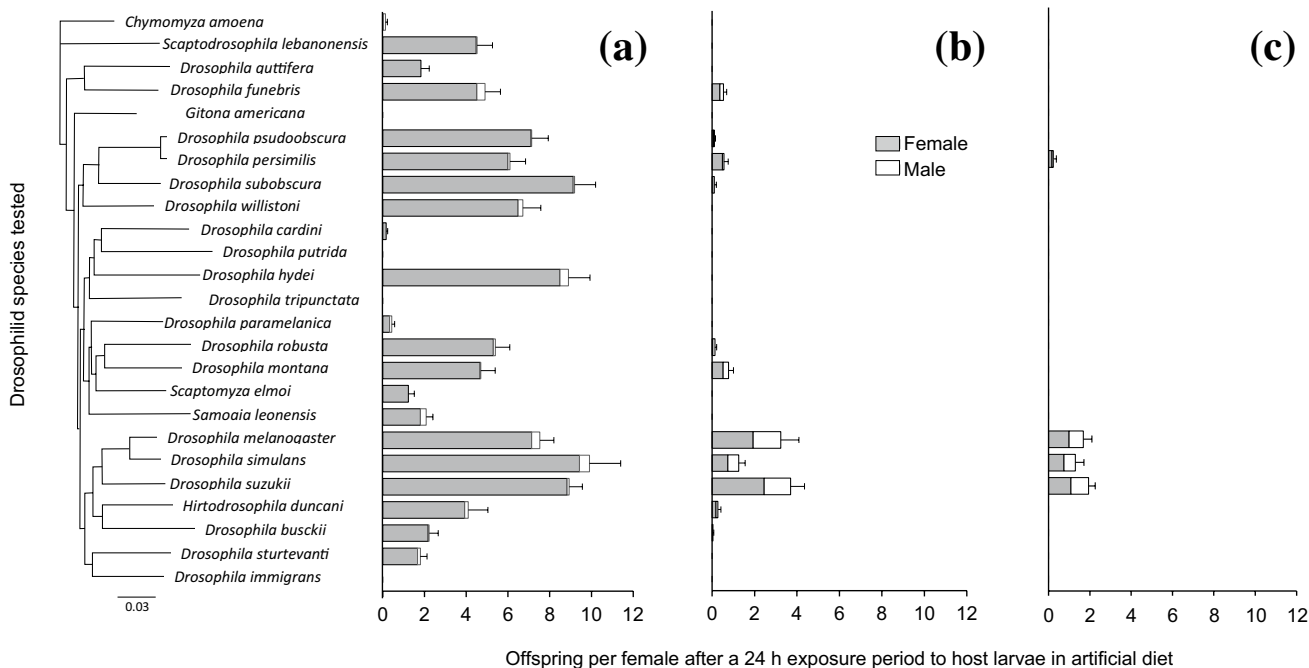
All three parasitoid species were able to parasitize *D. suzukii* under quarantine conditions, but the number of parasitized non-target host species varied greatly (Fig. 1). *Asobara japonica* developed from 19 out of 24 tested non-target species, including 16 of the 19 tested *Drosophila* spp. as well as species in the genera *Chymomyza*, *Hirtodrosophila*, *Samoia*, *Scaptodrosophila*, and *Scaptomyza*. As mentioned, *S. elmoi* represented endangered Hawaiian species. *Leptopilina japonica* developed from 9 non-target species; except for the tested *Hirtodrosophila* sp., all were *Drosophila* spp. and most produced <0.5 adult offspring per female per 24 h exposure period, compared with 3.2 and 3.7 from *D. melanogaster* and *D. suzukii*, respectively. For example, only 1, 2 and 3 *L. japonica* individuals (out of 600 exposed larvae for each species) developed from *D. robusta* Surtervant, *D. pseudoobscura* (Frolova and Astaurov) and *D. subobscura* Collin, respectively. *Ganaspis brasiliensis* developed from only three non-target species and two of these were *D. melanogaster* and *D. simulans*, both closely related to *D. suzukii*; only five *G. brasiliensis* individuals (of 600 exposed) emerged from the other non-target *D. persimilis*. All non-target species attacked by *G. brasiliensis* were also attacked by *L. japonica* (Fig. 1). Overall, more offspring were produced by *A. japonica* than by *G. brasiliensis* or

*L. japonica* on *D. suzukii* and on the non-target hosts from which all three parasitoids emerged.

Both host and parasitoid species affected the parasitoids’ performance measurements—degree of parasitism (DP), success rate of parasitism (SP), encapsulation rate (ER) and the number of parasitoid offspring that emerged (Table 1, Fig. 2). For SP, DP and ER, the maximum rate is ‘1’ for each performance measure, but with the stacked bars shown in Fig. 2, the highest value would be ‘2’ (e.g.,

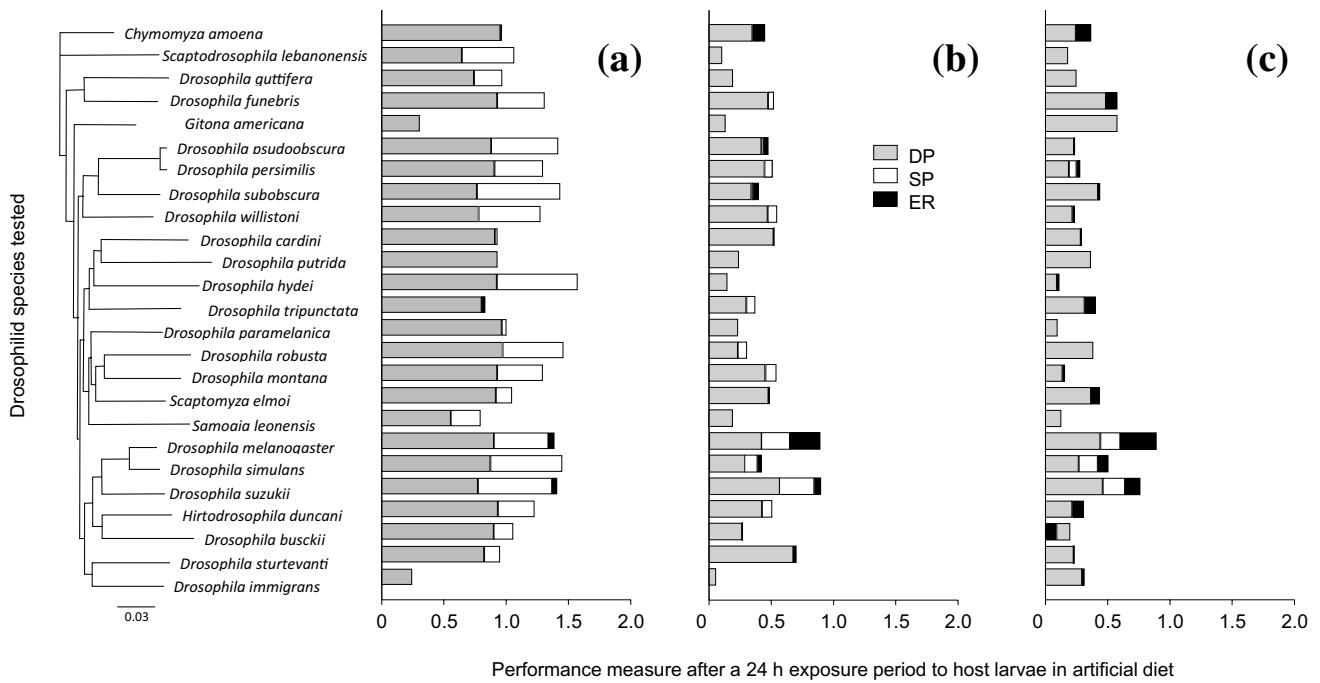
**Table 1** Results of GLMs analyzing the effects of parasitoid and host species on parasitoid performance

Performance parameter	Source	Df	$\chi^2$	P
Degree of parasitism (DP)	Parasitoid species (P)	2	515.1	<0.001
	Host species (H)	24	10.9	<0.001
	P×H	48	8.2	<0.001
Success rate of parasitism (SP)	Parasitoid species (P)	2	763.8	<0.001
	Host species (H)	24	46.8	<0.001
	P×H	48	15.7	<0.001
Encapsulation rate (ER)	Parasitoid species (P)	2	29.9	<0.001
	Host species (H)	24	16.5	<0.001
	P×H	48	3.0	<0.001
Number of wasps emerged	Parasitoid species (P)	2	633.9	<0.001
	Host species (H)	24	42.2	<0.001
	P×H	48	19.2	<0.001



**Fig. 1** Mean (± SE) number of offspring that emerged as adult wasps after (a) *A. japonica*, (b) *L. japonica* and (c) *G. brasiliensis* adult females were exposed to drosophilid host larvae (exposed in diet) for a 24 h period, with host species arranged in a phylogenetic tree con-

structed based on COI gene sequences. Values were analyzed using a two-way analysis of variance on the effects of parasitoid species, host species and their interaction (see Table 1)



**Fig. 2** Performance of (a) *A. japonica*, (b) *L. japonica* and (c) *G. brasiliensis* on various host species based on the Degree of Parasitism (DP), Success Rate of Parasitism (SP), and Encapsulation Rate (ER), with host species arranged in a phylogenetic tree constructed based

on COI gene sequences; the four host species successfully parasitized by *G. brasiliensis* are highlighted by the gray bar. Values are means and were subjected to a two-way analysis of variance on the effects of parasitoid species, host species and their interaction (see Table 1)

for 100% SP and DP, must have 0% ER, conversely 100% ER and DP must have 0% SP). Overall, DP and SP were higher for *A. japonica* than for *L. japonica* or *G. brasiliensis* (Fig. 2). Encapsulation of parasitoids occurred in only 5 host species for *A. japonica*, but in 13 host species for *L. japonica* and 18 host species for *G. brasiliensis* (Fig. 2, Fig. S2). None of the tested parasitoid performance parameters (DP, SP, ER, number of offspring per day per female) were associated with fly phylogeny for *A. japonica*, whereas significant phylogenetic signals were detected for SP and number of offspring for *L. japonica* and *G. brasiliensis*, and also for ER for *G. brasiliensis* (Table 2).

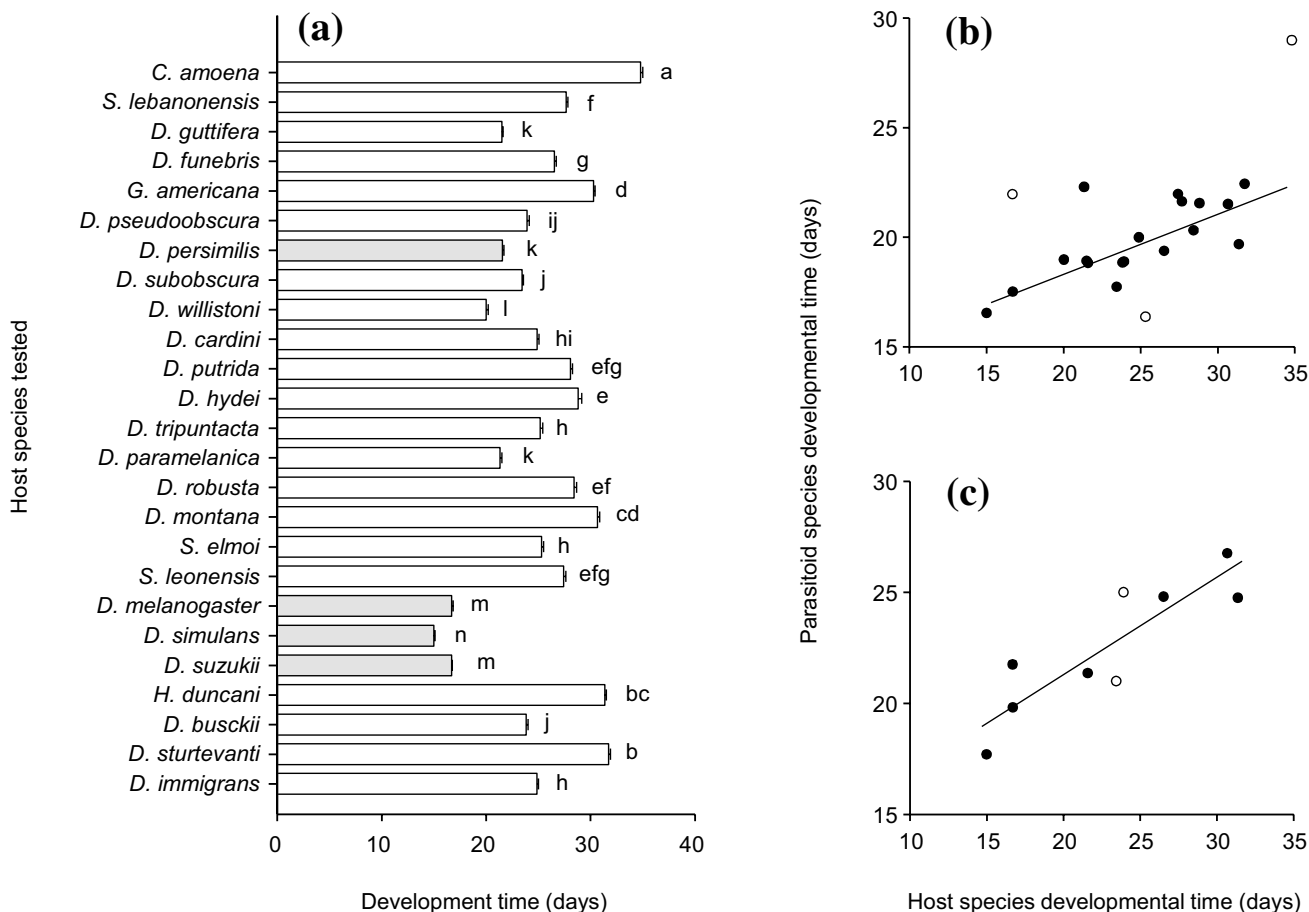
Preimaginal periods varied largely among various drosophilid species, ranging from 14.6 days in *D. simulans* to 34.8 days in *C. amoena* (Loew) ( $F_{24,5463} = 838.6, P < 0.001$ )

(Fig. 3a) and the developmental time of both *A. japonica* (Fig. 3b) and *L. japonica* (Fig. 3c) increased with the host developmental time. Based on the developmental time on *D. sukuzii*, *A. japonica* ( $17.5 \pm 0.22, n = 108$ ) developed faster than *L. japonica* ( $19.8 \pm 0.34, n = 130$ ) and *L. japonica* developed faster than *G. brasiliensis* ( $22.4 \pm 0.22, n = 84$ ) ( $F_{2,319} = 64.5, P < 0.001$ ).

The body size of host species varied among the 16 measured *Drosophila* species, in terms of pupal length ( $F_{15,379} = 984.1, P < 0.001$ ), pupal width ( $F_{15,379} = 169.6, P < 0.001$ ) and pupal volume ( $F_{15,379} = 377.4, P < 0.001$ ) (Table 3). Adult *A. japonica* females that emerged from larger host species had larger body sizes (hind tibia:  $F_{15,379} = 62.9, P < 0.001$ ; ovipositor length  $F_{15,379} = 45.7, P < 0.001$ ) and higher mature egg loads ( $F_{15,379} = 20.2,$

**Table 2** Phylogenetic signal in parasitoid performance parameters as measured by Moran’s *I*;  $P < 0.05$  is the probability that a performance measure for *A. japonica*, *L. japonica* or *G. brasiliensis* is influenced by phylogeny of the non-target species tested

Parameter	<i>A. japonica</i>		<i>L. japonica</i>		<i>G. brasiliensis</i>	
	<i>I</i>	<i>P</i>	<i>I</i>	<i>P</i>	<i>I</i>	<i>P</i>
Degree of parasitism (DP)	−0.037	0.382	−0.034	0.392	−0.036	0.350
Success rate of parasitism (SP)	−0.019	0.213	−0.017	0.028	−0.008	0.041
Encapsulation rate (ER)	−0.035	0.163	−0.041	0.503	−0.014	0.048
Offspring per day per female	−0.005	0.104	−0.015	0.016	−0.001	0.024



**Fig. 3** (a) Immature developmental time of the tested drosophilid host species (values are mean  $\pm$  SE and bars bearing different letters are significantly different, Tukey's HSD,  $P < 0.05$ ) and the corresponding relationship of mean developmental time for (b)

*A. japonica* ( $y = 0.291x + 12.365$ ,  $r^2 = 0.486$ ) and (c) *L. japonica* ( $y = 0.423x + 12.842$ ,  $r^2 = 0.847$ ) on those hosts that were attacked and were successfully parasitized (open circles are for hosts with <5 offspring and were not included in the regression analyses)

$P < 0.001$ ) (Table 3). Ovipositor length was positively related to hind tibia length in female *A. japonica* (ovipositor =  $0.320 + 0.520 \times$  hind tibia;  $F_{15,379} = 45.7$ ,  $P < 0.001$ ,  $R^2 = 0.635$ ,  $P < 0.001$ ). Overall, mean body size of *A. japonica* females increased with mean host size ( $y = 0.928x + 1.311$ ,  $R^2 = 0.568$ ,  $P < 0.001$ ) and mean female mature egg load increased with the mean body size of female wasps ( $y = 62.15x - 33.63$ ,  $R^2 = 0.695$ ,  $P < 0.001$ ).

**Discussion**

This study showed clear differences in the physiological host ranges of *A. japonica*, *G. brasiliensis* and *L. japonica* populations collected from South Korea. Results indicate that *G. brasiliensis* was the most host specific; *L. japonica* could develop on a wider host range; and *A. japonica* developed from most of the 25 tested drosophilid species regardless of their phylogenetic position. All three parasitoids were able

to parasitize various host larvae although success levels were generally higher for *A. japonica* than for *G. brasiliensis* or *L. japonica* as measured by offspring production and degree of parasitism and successful parasitism.

These data are critical to define the physiological host range and to determine suitability for use in a classical biological control program, although this may not translate directly into ecological suitability and performance in a pest's invaded range, as the ecological host range may further limit a parasitoid's host range in the field. Therefore, ascertaining a parasitoids' host range and specificity in no-choice, small cage studies, such as those conducted within the confines of a quarantine, is a conservative approach to measuring risk posed by imported natural enemies (van Lenteren et al. 2006). As reviewed in Hoddle et al. (2020), no-choice experiments may overestimate the number of species attacked by a natural enemy species (i.e., fundamental or physiological host range) as the natural enemy is confined with hosts that it may not normally encounter or that

**Table 3** Pupal sizes of various *Drosophila* species and body size and mature egg load of female *A. japonica*

Host species	Host pupal size (mm <sup>3</sup> ) <sup>a,b</sup>	Wasp body size (mm) and egg load <sup>a</sup>		
		Hind tibia length	Ovipositor length	Mature egg load
<i>D. busckii</i>	0.321 ± 0.007 d	1.44 ± 0.02 bc	1.01 ± 0.011 ef	57.5 ± 2.4 defg
<i>D. funebris</i>	0.362 ± 0.013 cd	1.78 ± 0.29 a	1.28 ± 0.026 ab	71.7 ± 4.2 bcde
<i>D. guttifera</i>	0.121 ± 0.004 hi	1.46 ± 0.03 bc	1.11 ± 0.024 cd	62.4 ± 2.7 cdef
<i>D. hydei</i>	0.679 ± 0.014 a	1.84 ± 0.03 a	1.35 ± 0.018 a	94.3 ± 3.6 a
<i>D. melanogaster</i>	0.165 ± 0.005 fg	1.27 ± 0.02 d	0.99 ± 0.024 f	46.4 ± 3.9 fg
<i>D. montana</i>	0.379 ± 0.010 c	1.83 ± 0.02 a	1.20 ± 0.027 bc	77.9 ± 3.0 abc
<i>D. persimilis</i>	0.192 ± 0.008 efg	1.47 ± 0.02 b	1.05 ± 0.006 def	49.7 ± 2.1 fg
<i>D. pseudoobscura</i>	0.177 ± 0.004 fg	1.54 ± 0.03 b	1.09 ± 0.016 de	55.1 ± 2.9 efg
<i>D. robusta</i>	0.550 ± 0.018 b	1.76 ± 0.03 a	1.26 ± 0.016 ab	73.0 ± 5.5 bcd
<i>D. simulans</i>	0.153 ± 0.004 gh	1.56 ± 0.02 b	1.13 ± 0.017 cd	62.1 ± 2.8 cdef
<i>D. subobscura</i>	0.204 ± 0.009 ef	1.73 ± 0.01 a	1.25 ± 0.015 b	78.7 ± 3.5 abc
<i>D. suzukii</i>	0.233 ± 0.010 e	1.49 ± 0.04 b	1.23 ± 0.022 b	55.2 ± 4.4 efg
<i>D. willistoni</i>	0.102 ± 0.005 i	1.33 ± 0.02 cd	0.98 ± 0.016 f	44.0 ± 2.8 g
<i>H. duncani</i>	0.163 ± 0.004 fgh	1.55 ± 0.03 b	1.11 ± 0.014 cd	81.0 ± 2.9 abc
<i>S. elmoi</i>	0.054 ± 0.002 j	1.11 ± 0.02 e	0.87 ± 0.020 g	42.1 ± 2.5 g
<i>S. lebanonensis</i>	0.179 ± 0.004 fg	1.57 ± 0.03 b	1.10 ± 0.022 cd	47.8 ± 4.6 fg

<sup>a</sup>Values (mean ± SE) followed by different letters are significantly different within each column ( $P < 0.05$ )

<sup>b</sup>Host pupal size or volume was determined by measurements of pupal length and width

it would ignore or abandon if found in nature. The physiological host range is often much larger than the ‘ecological’ host range of the arthropod natural enemy under evaluation (Heimpel and Mills 2017). Quarantine studies are then, to some extent, a relative measure of the ecological risk of the tested natural enemy species. For example, *A. japonica* is likely an effective *D. suzukii* parasitoid but also appears from our Quarantine tests to be a greater non-target threat. This is backed up by field surveys (e.g., ecological host range) that show *A. japonica* attacks not only *Drosophila* spp. but also species in the genera or subgenera *Chymomyza*, *Dorsilopha*, *Scaptodrosophila* and *Sophophora* (Ideo et al. 2008; Mitsui and Kimura 2010; Mitsui et al. 2007).

In contrast, the two figitids had narrower physiological host ranges, especially the more host-specific populations of *G. brasiliensis* (G1 and G3) (Daane et al. 2016; Giorgini et al. 2019; Girod et al. 2018b; Matsuura et al. 2018; Nomano et al. 2017; Seehausen et al. 2021). Moreover, host phylogeny strongly influenced the success rate of parasitism in both figitids. We found that the South Korean *L. japonica* readily developed from the three *melanogaster* species group members (*D. melanogaster*, *D. simulans* and *D. suzukii*), and only a few individuals emerged from another 7 species. Girod et al. (2018b) reported similar results for the two Chinese *L. japonica* populations; both successfully parasitized *D. melanogaster* and *D. subobscura*, with only one progeny emerging from *D. immigrans*. In Japan, *L. japonica* was collected almost entirely from cherry fruit infested by *D. suzukii* (Matsuura et al. 2018), while in China *L. japonica* was collected from fresh fruits infested by *D. pulchrella* Takamori

and Watabe, *D. subobscura*, and *D. suzukii*, (which also possess a serrated ovipositor that allows it to penetrate the intact skin of fruits) (Giorgini et al. 2019), while in South Korea it was collected from fresh fruits infested by *D. suzukii* (Daane et al. 2016). Kimura and Novkovic (2015) found that the *L. japonica* population was a highly efficient parasitoid of some *Drosophila* species from its original locality, but was less successful on species from other localities. However, the host range of this parasitoid appears to be largely limited to the *melanogaster* species group. The *G. brasiliensis* population we tested had an even narrower physiological host range that included only the closely related *D. melanogaster*, *D. simulans*, and *D. suzukii*, with a small fraction attacked of the more distantly related *D. persimilis*, suggesting that little to no non-target impact in the field would occur.

There is some plasticity in these parasitoids’ responses to different host species. For example, larval development time varied considerably among the 25 tested drosophilid species. Both *A. japonica* and *L. japonica* seemed to adjust their larval development rate to that of their hosts (Fig. 3). These results agree with Kohyama et al. (2017) who showed that *A. japonica* and *Leptopilina ryukyensis* Novkovic & Kimura retarded their own development before host pupariation. The retardation of development time is likely advantageous in koinobiont parasitoids because excessive development of parasitoid larvae before host pupariation would have negative effects on host development and eventually on parasitoid fitness (Harvey and Strand 2002). *Asobara japonica* also displayed plasticity in female body size, which increased with host size. Body size is often positively correlated



with many other fitness components such as adult longevity, fecundity, foraging efficiency and mating ability (e.g., Wang and Messing 2004). As expected, mature egg load also increased with female body size. Such developmental plasticity may contribute to the persistence of *A. japonica* under variable conditions.

A critical aspect of this work is the acknowledgement that host specificity may vary among geographical populations of *G. brasiliensis* to the extent that populations studied may represent cryptic species. We report herein that a South Korean *G. brasiliensis* population parasitized *D. melanogaster*, *D. simulans*, and *D. suzukii* and, to a lesser extent, *D. persimilis*. Girod et al. (2018b) report a Japanese population of *G. cf. brasiliensis* was strictly specific to *D. suzukii*, whereas a population from China parasitized *D. melanogaster*, *D. suzukii* and, to a lesser extent, *D. subobscura*. Giorgini et al. (2019) tested a *G. brasiliensis* population from China on 9 of the 25 host species tested in this study and found similar host relationships as reported herein. A first attempt to understand *G. brasiliensis* population differences and host relationships was made by Nomano et al. (2017), who grouped *G. brasiliensis* into five lineages (G1–G5) based on molecular analyses of the cytochrome oxidase subunit 1 (CO1) gene and three nuclear DNA regions (ITS1, ITS2 and RpL37). However, Buffington and Forshage (2016) found different lineages were morphologically indistinguishable. Moreover, a recent phylogenomic reconstruction on a representative group of the G1–G5 lineages using a large (ca. 1300 loci) ultraconserved element (UCE) data set showed differences among *G. brasiliensis* populations, but COI-assignments were intermingled and not monophyletic (Buffington et al. unpubl. data). Still, to reference different studies we will use the G1–G5 designations and our discussion will focus on only the G1 and G3 lineages. Our South Korean populations reported herein were a mixture of G1 and G3 lineages, based on CO1 analyses of voucher specimens. Populations of *G. brasiliensis* collected from Yunnan, China, also found a mixture G1 and G3 specimens, and genetic distance, based on the COI gene, was large enough (5–7%) to suggest reproductive isolation (Giorgini et al. 2019). Moreover, G1 was the dominant lineage (> 65%) reared from *D. suzukii* in both the South Korean and Chinese collections processed at the Berkeley quarantine, and the lineages were sympatric and co-existed on the same host plants inhabited by *D. suzukii* (in South Korea) and by *D. suzukii* and/or *D. pulchrella* or *D. subpulchrella* Takamori and Watabe (in China). Other researchers reported that G1 populations collected from *D. suzukii*-infested cherries in Japan specialize on *D. suzukii* (Girod et al. 2018b; Matsuura et al. 2018; Nomano et al. 2017). Seehausen et al. (2021) suggest G1 and G3 are, in fact, cryptic species that can be differentiated by acid-soluble insect protein spectra, some host-searching behaviors, and incompatible crossing.

Therefore, based on our molecular examination of voucher specimens from the current study, and the discussion above, we suggest the populations studied herein were a combination of G1 and G3 with the possibility that G1 attacked only *D. melanogaster* and *D. suzukii* (a G1 voucher specimen was reared from *D. melanogaster*) and that G3 attacked *D. melanogaster*, *D. simulans*, and *D. suzukii*, and a small number of *D. persimilis*.

We also note that the figitids attacked more host species than they were able to successfully develop from, as indicated by the encapsulation rate (Fig. 2). In fact, there was a moderately high encapsulation rate recorded for both *G. brasiliensis* or *L. japonica* attacking *D. melanogaster* and *D. suzukii*, but rarely in *A. japonica*. We further suggest that although the G3 lineage tested in the current study can readily attack *D. melanogaster* and *D. simulans*, we would not expect it to significantly impact these non-target species if it were released. First, both *G. brasiliensis* G1 and G3 populations from South Korea and China were found mainly on various fresh fruits, not in over-ripe or rotted fruit that other drosophilid species such as *D. melanogaster* and *D. simulans* favor; larvae of these species are attacked by a number of braconid and other figitid parasitoids that do not attack *D. suzukii*, further suggesting that the parasitoid communities attacking drosophilids are partitioned by microhabitat. In previous experiments we showed that *G. brasiliensis* was outcompeted by *L. japonica* and believe that competition may have driven *G. brasiliensis* to specialize on *D. suzukii* and other drosophilids (e.g., *D. pulchrella*) that attack ripening fruit (Wang et al. 2019a). *Drosophila melanogaster* and *D. simulans* are two common cosmopolitan species that occur in a wide range of habitats, and they likely overlap with *D. suzukii* when fresh fruits become unavailable to *D. suzukii* (e.g., Wang et al. 2016). *Ganaspis brasiliensis* would likely encounter competition in these alternative fly hosts from the diverse complex of larval parasitoids attacking *D. melanogaster* and *D. simulans* on rotting fruit, including *A. tabida*, *L. heterotoma* and *L. bouvardi* (Carton et al. 1986; Chabert et al. 2012; Kacsoh and Schlenke 2012; Miller et al. 2015). *Ganaspis brasiliensis* might attack *D. melanogaster* or *D. simulans* when ripe fruit is no longer available and *D. suzukii* is rare. This could help persistence and increase population sizes and/or enhance their activity in crop systems and may eventually lead to an increased impact on *D. suzukii* populations. It is also possible that any introduced *D. suzukii* parasitoids would prefer to attack *D. suzukii* for a “competition-free space”, to avoid competition with other larval parasitoids on *D. melanogaster* and *D. simulans* (Messing and Wang 2009).

We conclude that *G. brasiliensis* should be considered for release from quarantine for the classical biological control of *D. suzukii* in North America. This conclusion builds upon an earlier study in Europe (Girod et al. 2018b) of *G. brasiliensis*

(referred to as *G. cf. brasiliensis*) populations from Yunnan, China, and Tokyo, Japan; *L. japonica* populations from Yunnan and Beijing, China; and an *A. japonica* population from Tokyo, Japan. They used *D. busckii* Coquillett, *D. hydei* Sturtevant, *D. immigrans* Sturtevant, *D. melanogaster* and *D. subobscura* as non-target species (these fly species were also tested in this study). Girod et al. (2018b) similarly found that *G. brasiliensis* was the most host specific, followed by *L. japonica*, and then *A. japonica*, the latter of which developed from all five non-target species—although there was a lower success rate on *D. immigrans* (1.2%). Similarly, we found *A. japonica* attacked these hosts, but that no *A. japonica* emerged from *D. immigrans*. This difference may have resulted from population differences (South Korean vs. Japan) as *A. japonica* was reported to parasitize *D. immigrans* in field collections in Japan (Ideo et al. 2008), but no evidence for such host-parasitoid field association in South Korea is available. We therefore suggest that *A. japonica* should not be considered for release, even though it is an effective parasitoid of *D. suzukii* it presents too great of a non-target risk based on its physiological host range.

## Author contributions

AB, XG and KMD conceived and designed experiments; KMD obtained funding; AB and XG collected data; XG, BNH and AB analyzed data; all authors contributed critically to the submitted draft.

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**Availability of data and material** Data available from the Dryad Digital Repository (to be provided on acceptance of manuscript).

**Code availability** Not applicable.

## Declarations

**Conflict of interest** The authors declare that they have no competing interests.

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