BioControl (2019) 64:263–275
https://doi.org/10.1007/s10526-019-09933-5

Life history of *Vrestovia fidenas*, a potential control agent of *Drosophila suzukii*

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Received: 20 August 2018 / Accepted: 26 March 2019 / Published online: 1 April 2019

Abstract *Vrestovia fidenas* (Walker) (Hymenoptera: Pteromalidae) is a pupal parasitoid of *Drosophila* spp. native to Europe and could be useful for the biological control of the invasive spotted-wing Drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae). We assessed life-table parameters (fecundity and longevity of adults, and weight and developmental time of offspring) of *V. fidenas* on two hosts: *D. suzukii* and the common native vinegar fly, *Drosophila subobscura* Collin (Diptera: Drosophilidae). *Drosophila suzukii* was determined to be a suitable host for *V. fidenas* and a better host than *D. subobscura*, i.e., *V. fidenas* produced more offspring and developed more rapidly on *D. suzukii* than on *D. subobscura*. The results indicate that *V. fidenas* should be further assessed for the biological control of *D. suzukii*. In addition, morphological analysis demonstrated that a second species of the genus *Vrestovia*, *V. brevior*, is also present in Europe. We present an identification key to the most common pteromalid pupal parasitoids of *Drosophila*, including both species of *Vrestovia*.

Keywords *Trichopria* · *Spalangia* · *Pachycrepoideus* · *Vrestovia brevior* · *Drosophila melanogaster* · Switzerland · Europe · New record

Handling Editor: Dirk Babendreier.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10526-019-09933-5) contains supplementary material, which is available to authorized users.

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Introduction

Since 2008, a vinegar fly native to Southeast Asia, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), has been spreading across Europe and the Americas, infesting ripening fruit and berries and causing substantial losses in production (Asplen et al. 2015). Much research has been conducted in the invaded areas to identify native natural enemies that may have formed new associations with *D. suzukii* for the biological control of this invasive insect in agriculture (Cuthbertson et al. 2014; Gabarra et al.

2015; Knoll et al. 2017; Mazzetto et al. 2016; Miller et al. 2015; Wolf et al. 2018). Most attention has been given to the hymenopteran parasitoids *Trichopria drosophilae* (Perkins) (Hymenoptera: Diapriidae) and *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae) (Chabert et al. 2012; Rossi Stacconi et al. 2015, 2018). Another hymenopteran parasitoid of *Drosophila* spp. and a potential biological control agent of *D. suzukii* is *Vrestovia fidenas* (Walker) (Hymenoptera: Pteromalidae), which was recorded during a field survey in Switzerland (Knoll et al. 2017). *Vrestovia fidenas* is a pupal parasitoid and is currently the only member of its genus known from Europe (Bouček 1965; Noyes 2018).

Parasitoid wasps in the genus *Vrestovia* are known to attack *Drosophila* spp. (Bouček 1993; Offenberger and Klarenberg 1997; Thistlewood et al. 2013), and two species, *V. fidenas* and *Vrestovia brevior* Bouček (Hymenoptera: Pteromalidae), can fully develop in *D. suzukii* (Knoll et al. 2017; Thistlewood et al. 2013). The known distribution of *V. fidenas* is Europe and the Caucasus, whereas *V. brevior* has only been described for North America (Noyes 2018). The biology of the two species is not known so far, but such information is required in order to assess their potential as biological control agents.

Another important requirement before parasitoids are released in control programs is their correct identification, which is often difficult to achieve even for experts. However, identification is necessary to understand the biology and ecology of related and/or morphologically similar species and is therefore necessary for the licencing of potential biological control agents (Bigler et al. 2005; Mason et al. 2017).

The present study was initiated to determine the life history of *V. fidenas* on two hosts, the common and native *Drosophila subobscura* Collin (Diptera: Drosophilidae) and the invasive *D. suzukii.* We compared the adult parasitoid's fecundity and longevity, as well as the sex, weight, and developmental time of offspring on both hosts. For the identification of the most common pteromalid parasitoids of *Drosophila*, we also developed a morphological key to the genus level and to the species level for the genus *Vrestovia.* We demonstrate that females of *V. fidenas* can parasitize pupae of *D. suzukii* and that *V. brevior* is also present in Europe.

Materials and methods

Insects

The cultures of *D. suzukii* and *D. subobscura* (approximately 70–90 generations had been reared at the time of the experiments) originated from individuals collected in Zürich, Switzerland, in 2013 (Knoll et al. 2017). *Drosophila subobscura* was chosen as a host for the following reasons: (1) it is among the most common *Drosophila* species in Switzerland (Bächli and Burla 1985); (2) it is similar to *D. suzukii,* i.e., both species are frugivorous and can be reared on the same diet and under the same environmental conditions; and (3) *V. fidenas* was not exposed to *D. subobscura* during rearing, and potential adaptation could therefore be excluded. *Drosophila subobscura* is a palearctic species that invaded North and South America (Prevosti et al. 1988) but is not present in Asia (Bächli 2018). A wild-type *Drosophila melanogaster* Meigen culture that was used for rearing the parasitoids was obtained from the University of Basel, Switzerland. All three *Drosophila* spp. were reared on an artificial diet (1600 g of peeled banana, 200 g of brewer's yeast, 120 g of flour, 80 g of sugar, 80 g of agar, 16 g of nipagin diluted in 120 ml of EtOH [70%], and 2120 ml of water) that served as both a food source and an oviposition substrate. The diet was changed regularly, i.e., the diet was changed three times per week for *D. melanogaster*, and was exchanged daily during the experiments for *D. suzukii* and *D. subobscura*. The diet that contained the *Drosophila* eggs was placed into 0.8 l plastic vials containing fresh diet as food source for the developing larvae. The last stage larvae of *D. suzukii* and *D. subobscura* were used for the experiments (see below).

The *V. fidenas* culture was initiated with a few individuals collected in Altnau (Thurgau, Switzerland) in 2015 (approximately 20–30 generations had been completed at the time of the experiments) in sentinel traps containing pupae of *D. melanogaster* (Knoll et al. 2017). Specimens were reared in a flight cage (32 × 22 × 16 cm) and provided with water and honey *ad libitum. D. melanogaster* pupae were offered on dental cotton rolls to adult parasitoids for oviposition. The rolls were replaced with new rolls two times per week, and the parasitized pupae were held until parasitoid emergence. Emerged female and male *V. fidenas* were held together in 250 ml plastic

vials containing honey to allow mating. The insects were reared and the experiments were conducted in climate chambers at 22 °C, 70% RH, and 16:8 L:D.

Experiments

Determination of pupal weight

To determine the amount of food resources available for the development of parasitoid larvae in host pupae, we weighed the pupae of the two hosts. Pupae of *D. suzukii* and *D. subobscura* were collected by placing a piece of moistened paper towel (10 × 20 cm) into a vial with last stage *Drosophila* larvae. Many of the last stage larvae pupated on the paper towel within 24 h, at which time the towel, with both pupae and those last stage larvae that had not yet pupated, was placed in an empty vial that was covered with plastic gauze (to ensure air flow). The latter vial was kept in the climate chamber for another 24 h to allow all larvae to pupate. Because some larvae had pupated shortly after the paper towel was introduced to the first vial but others did not pupate until after the paper towel was moved to the second vial, pupae were estimated to be 0–48 h old at the time of weighing. Pupae were carefully removed from the paper towel with a brush and rinsed in tap water to remove traces of the diet. The pupae were dried at room temperature for approximately 30 min and then individually weighed (Mettler Toledo MX5 microbalance; ± 0.002 mg). Two and three separate batches of pupae were produced and weighed for *D. suzukii* and *D. subobscura*, respectively.

Life-table parameters

Males and females (F0 generation) of *V. fidenas* that had hatched within the previous 24 h were randomly assigned to pupae of either *D. suzukii* or *D. subobscura* as host species for oviposition. One pair (i.e., one male and one female) of the parasitoid was placed in a 60 ml plastic vial containing a moist cotton pad, a drop of honey, and 20 pupae of one of the *Drosophila* host species. The parasitoid pairs were transferred daily to a new vial containing the same resources until the parasitoids died. Whenever a male died, it was replaced with a newly hatched male to ensure that the female could mate at all times. When a female died, the male was offered honey and a moist cotton pad until it died. The pupae of *D. suzukii* and *D. subobscura* that were added to the 60 ml vials each day were obtained by using paper towels as described above, except that all pupae were ≤ 24 h old when added. The experiment was repeated with four different *V. fidenas* cohorts, each time with five replicate pairs of the parasitoid and with five controls (pupae only) per *Drosophila* host species. All pupae were held until emergence of flies or parasitoids. The number and sex of *V. fidenas* offspring and the number of flies that emerged were recorded daily. Developmental time of *V. fidenas* was calculated from the day pupae were parasitized until parasitoid emergence. Emerged *V. fidenas* offspring were collected daily and stored in 96% EtOH plastic tubes (with one tube per parasitoid pair). The dry weight of the offspring was measured as a proxy for offspring fitness (Honěk 1993). To prepare the offspring for weighing, the EtOH was removed, and a piece of filter paper was added to each tube to facilitate evaporation of residual EtOH. The open tubes were then kept in an oven at 40 °C for 2 h and thereafter at room temperature for 22 h. Dried wasps were then individually weighed (Mettler Toledo MX5 microbalance; ± 0.002 mg). For each parasitoid pair, the proportion of female offspring was calculated as the number of females divided by the total number of offspring (females and males), without considering the offspring of females that produced only males. The longevity of the F0 generation of *V. fidenas* was calculated from the date of emergence to the date of death. In addition, the number of days during which offspring were produced was considered as the oviposition period.

Survival of male V. fidenas with or without access to females

Within 24 h after emergence, male *V. fidenas* were randomly assigned to one of the following three treatments: one male with no female, one male with one female, or one male with three females. The *V. fidenas* females were < 24 h old. The specimens were kept in 60 ml plastic vials containing a moist cotton pad and a drop of honey. Survival was checked daily. If a female died, it was replaced with a freshly emerged female. Each treatment was represented by two to six vials, and the experiment was performed four times, so that a total of 18–21 males per treatment were assessed.

Life history strategy

To determine whether *V. fidenas* is an endo- or ecto-parasitoid, and whether it is solitary or gregarious, pupae of *D. suzukii* on paper towels were prepared as described above. Five *V. fidenas* females that had been held together with males to allow mating were offered 40 *D. suzukii* pupae in a 250 ml vial provided with honey. After 24 h, the pupae were frozen, and ten were randomly chosen and dissected and examined with a stereomicroscope (Zeiss Stemi SV11, Plan S 1.0× objective and 10× oculars) to determine the number of eggs or larvae per pupa and to determine whether eggs were laid on the surface of or within the pupa.

Statistical analysis

All statistical analyses were executed in R version 3.4.4 (R Core Team 2018). Pupal weight was compared between the *Drosophila* species with the Mann–Whitney test, which accounts for unequal sample sizes. For each species, the weight data from the different batches were pooled. To compare fly emergence from pupae between the *Drosophila* species in the control treatments of the life-table parameters experiment, a contingency table was tabulated and the Pearson's χ^2 test used (CrossTable: package *gmodels*, Warnes et al. 2018). Because the data were not normally distributed and heteroscedastic, robust statistical methods based on trimmed means were chosen for most of the remaining analyses (Wilcox 2012). The trimmed mean is a robust alternative to the arithmetic mean. A certain percentage (20% in our case) of the highest and lowest ordered scores is removed, then the arithmetic mean is computed on the remaining scores (Mair and Wilcox 2018b; Wilcox 2012). The one-way ANOVA for trimmed means uses an *F*-distributed test statistic with degrees of freedom that can have decimal numbers (t1way: package *WRS2*, Mair and Wilcox 2018a, for details see Mair and Wilcox 2018b). The two- and three-way ANOVAs for trimmed means use a test statistic "Q" that follows an χ^2-distribution, but with an adjusted critical value (needed for small sample sizes) that does not report degrees of freedom (t2way and t3way: package *WRS2*, for details see Mair and Wilcox 2018b and Wilcox 2012). Two-way ANOVAs for trimmed means were conducted to determine the effect of host species (*D. suzukii* vs. *D. subobscura*) and cohort (four independent cohorts) on the number of offspring, developmental time, adult weight, and proportion of females. Developmental time and weight were analysed separately for female and male offspring because males are usually smaller and develop faster than females of parasitic wasps. The effects of host species, sex, and cohort on longevity of parent parasitoids was analysed with a three-way ANOVA for trimmed means. The differences in oviposition period (days during which offspring were produced) between host species was analysed with a one-way ANOVA for trimmed means. The difference between survival curves of male *V. fidenas* alone or in presence of one or three females was analysed with the G-rho family (survdiff: package *survival*, Therneau 2015). The treatments were compared pairwise and the p-values adjusted with the method "BH" (pairwise_surfdiff: package *survminer*).

Morphology and species recognition

Nomenclature was used according to Noyes (2018). The terminology used for morphological characters follows Baur (2015). *Vrestovia fidenas* specimens from our cultures were killed in 96% EtOH, air dried, dissected, and mounted on cards according to Noyes (2018). Specimens of *Vrestovia* spp. from museum collections were also examined. All analysed specimens (voucher material) are listed in the Supplementary Data S1.

Specimens of *Vrestovia* spp. were photographed with a Keyence VHX 2000 digital photomicroscope and a VH-Z20R/W zoom lens at a magnification of 200× (i.e., 1000 µm corresponded to 888 pixels). A morphological key was developed for all pteromalid genera that have been reported to parasitize *D. suzukii* in Europe and North America (Kremmer et al. 2017; Kruitwagen et al. 2018), including the two *Vrestovia* species.

Results

Experiments

Pupal weight

The mean (± SE) weight per pupa was 1.82 mg ± 0.03 (n = 93) for *D. suzukii* and 1.41 mg ± 0.02

(n = 70) for *D. subobscura*. *D. suzukii* pupae weighed significantly more than *D. subobscura* pupae (Mann–Whitney test, W = 647, p ≤ 0.001).

Life-table parameters

In the absence of the parasitoid (i.e., in the control), 8337 flies emerged from 9275 *D. suzukii* pupae and 11,188 flies emerged from 12,360 *D. subobscura*. Fly emergence was not significantly influenced by the *Drosophila* species, χ^2(df = 1) = 2.40, p = 0.122).

Longevity was significantly longer for the F0 adults of *V. fidenas* that were offered pupae of *D. subobscura* for oviposition than for those that were offered *D. suzukii* pupae (t3way, Q = 7.42, p = 0.014; n = 78) (Fig. 1a, Table 1). There was also a significant interaction between host species and cohort (Q = 16.23, p = 0.015). Longevity was not significantly affected by parasitoid sex (Q = 3.27, p = 0.088) or parasitoid cohort (Q = 2.68, p = 0.52).

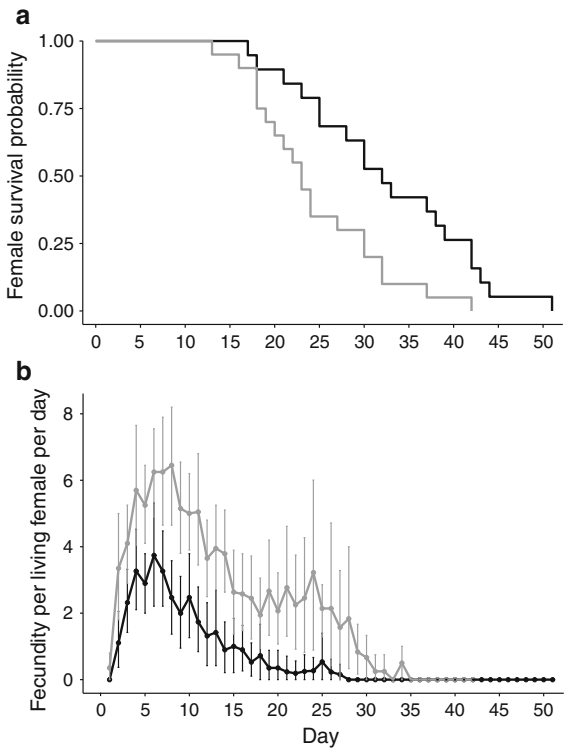

Fig. 1 Effects of *D. suzukii* (light line) vs. *D. subobscura* (dark line) pupae on **a** survival of *Vrestovia fidenas* females and **b** fecundity per living female per day (n = 19–20, means only include females that produced offspring). Fecundity was inferred from the number of offspring that emerged from parasitized pupae. The error bars show bootstrapped 95% confidence intervals of the means

Vrestovia fidenas females produced significantly more offspring (F1 adults) when offered *D. suzukii* pupae rather than *D. subobscura* pupae (t2way, Q = 8.54, p = 0.024; n = 39) irrespective of the cohort (Q = 0.92, p = 0.868) (Fig. 1b, Table 1). One female that did not produce offspring (from the *D. subobscura* treatment) was excluded from the analysis.

During the first day of exposure, parasitoid offspring were not produced from any *D. subobscura* pupae and from only 15% of the *D. suzukii* pupae. The peak of offspring emergence was from pupae exposed on day 6 with *D. subobscura* and on day 8 with *D. suzukii*. Thereafter, the number of offspring declined (Fig. 1b). The oviposition period of *V. fidenas* was approximately twice as long for females supplied with *D. suzukii* pupae than with *D. subobscura* pupae (mean ± SE of 18.25 ± 1.72 and 9.89 ± 1.41 days, respectively, t1way, F_t (df = 1, 22.98) = 13.46, p = 0.001) (Table 1). On the pupae of *D. subobscura*, the last offspring were produced on day 27, and the last female died on day 51. On *D. suzukii* pupae, in contrast, the last offspring were produced on day 34, and the last female died on day 42 (Figs. 1a, b). The proportion of female offspring was not significantly affected by host species (*D. suzukii* vs. *D. subobscura*) (Q = 0.13, p = 0.732; n = 35) or cohort (Q = 1.82, p = 0.69) (Table 1). *Vrestovia fidenas* females that produced only male offspring were not included in the analysis.

Female parasitoids developed faster on *D. suzukii* than on *D. subobscura* (Q = 61.22, p = 0.001; n = 1554) (Table 1). Female developmental time was also significantly affected by cohort (Q = 96.49, p = 0.001) and there was a significant interaction between host species and cohort (Q = 142.43, p = 0.001). Male parasitoid development was also faster on *D. suzukii* than on *D. subobscura* (Q = 24.59, p = 0.001; n = 792) and was significantly affected by cohort (Q = 93.36, p = 0.001). There was a significant interaction of host species and cohort (Q = 68.64, p = 0.001).

The weight per *V. fidenas* female was significantly higher when females developed on *D. suzukii* pupae rather than on *D. subobscura* pupae (Q = 153.69, p = 0.001; n = 326). Weight per female was also significantly affected by cohort (Q = 26.40,

Table 1 Life-table parameters (mean ± SE) of *Vrestovia fidenas* females and males on two hosts: *D. suzukii* and *D. subobscura*

Parameter	*D. suzukii*	*n*	*D. subobscura*	*n*	Statistics
Parasitoid females					
Longevity (d)	24.45 ± 1.66	20	32.45 ± 2.10	20	Q = 7.42, $\mathbf{p = 0.014}$[a]
Lifetime fecundity (no)	85.6 ± 11.67	20	34.11 ± 5.67	19	Q = 8.54, $\mathbf{p = 0.024}$
Oviposition period (d)	18.25 ± 1.72	20	9.89 ± 1.41	19	F_t = 13.46, $\mathbf{p = 0.001}$
Development time (d)	20.73 ± 0.05	1155	21.91 ± 0.09	399	Q = 61.22, $\mathbf{p = 0.001}$
Offspring dry weight (mg)	0.170 ± 0.0015	177	0.138 ± 0.0015	149	Q = 153.69, $\mathbf{p = 0.001}$
Proportion females[b]	0.69 ± 0.047	19	0.73 ± 0.039	16	Q = 0.13, p = 0.732
Parasitoid males					
Longevity (d)	28.63 ± 2.63	19	33.47 ± 2.33	19	Q = 7.42, $\mathbf{p = 0.014}$[a]
Development time (d)	18.59 ± 0.06	550	19.74 ± 0.12	242	Q = 24.59, $\mathbf{p = 0.001}$
Offspring dry weight (mg)	0.096 ± 0.0006	193	0.089 ± 0.0007	121	Q = 62.71, $\mathbf{p = 0.001}$

Longevity, lifetime fecundity, and oviposition period refer to the F0 generation; developmental time, proportion females, and offspring dry weight refer to the F1 generation. Differences between host species were analysed with ANOVAs for trimmed means (Mair and Wilcox 2018b) that either follow an F-distribution (one-way ANOVA) or an χ^2-distribution (test statistic Q in two- and three-way ANOVAs). Significant p values are shown in bold

[a]Longevity of males and females analysed together

[b]Offspring from females that produced both sexes: female offspring divided by total number of offspring

p = 0.001) (Table 1). The weight per *V. fidenas* male was also significantly higher when males developed on *D. suzukii* pupae rather than on *D. subobscura* pupae (Q = 62.71, p = 0.001; n = 314) (Table 1). Weight per male was not significantly affected by cohort (Q = 3.45, p = 0.347) (Table 1).

Survival of V. fidenas males with or without access to females

Males lived 38.71 ± 3.04 days (mean ± SE) with no female, 26.70 ± 3.26 days with one female, and 29.56 ± 2.32 days with three females. Male survival curves significantly differed between these three treatments (χ^2(df = 2) = 7.6, p = 0.022). Multiple comparisons showed that males without access to females survived significantly longer than males with one or three females (p = 0.027 for each comparison). Survival time did not significantly differ for males with access to one vs. three females (p = 0.897).

Life history strategy

The dissections revealed that one egg or larva of *V. fidenas* was present per pupa (five out of ten pupae were parasitized) and that the egg or larva was located within the *Drosophila* puparium and on the surface of the *Drosophila* pupa. These observations prove that *V. fidenas* is an ecto-parasitoid.

Morphology and species recognition

Key of the common pteromalid parasitoids of Drosophila

1. Antennal toruli in the lower face (below eyes) (Fig. 2c), antennal formula 11171, pronotum long (Fig. 2a)……..……***Spalangia***
   Antennal toruli in the upper face (Fig. 2d), antennal formula 11263 or 11353, pronotum short (Fig. 2b) ……..……**2**
2. Marginal vein thickened (Fig. 2e), malar space without malar depression (Fig. 2d) ……...……***Pachycrepoideus***
   Marginal vein not thickened (Fig. 2f), malar space with distinct malar depression (Fig. 4c/d) ……..…**3**
3. Antennal formula 11353, third anellus larger than the other two anelli (Fig. 4b) (if antennae damaged, go to 4)…….*Vrestovia brevior* female
   Antennal formula 11263 (Fig. 4a), both anelli small……..…**4**

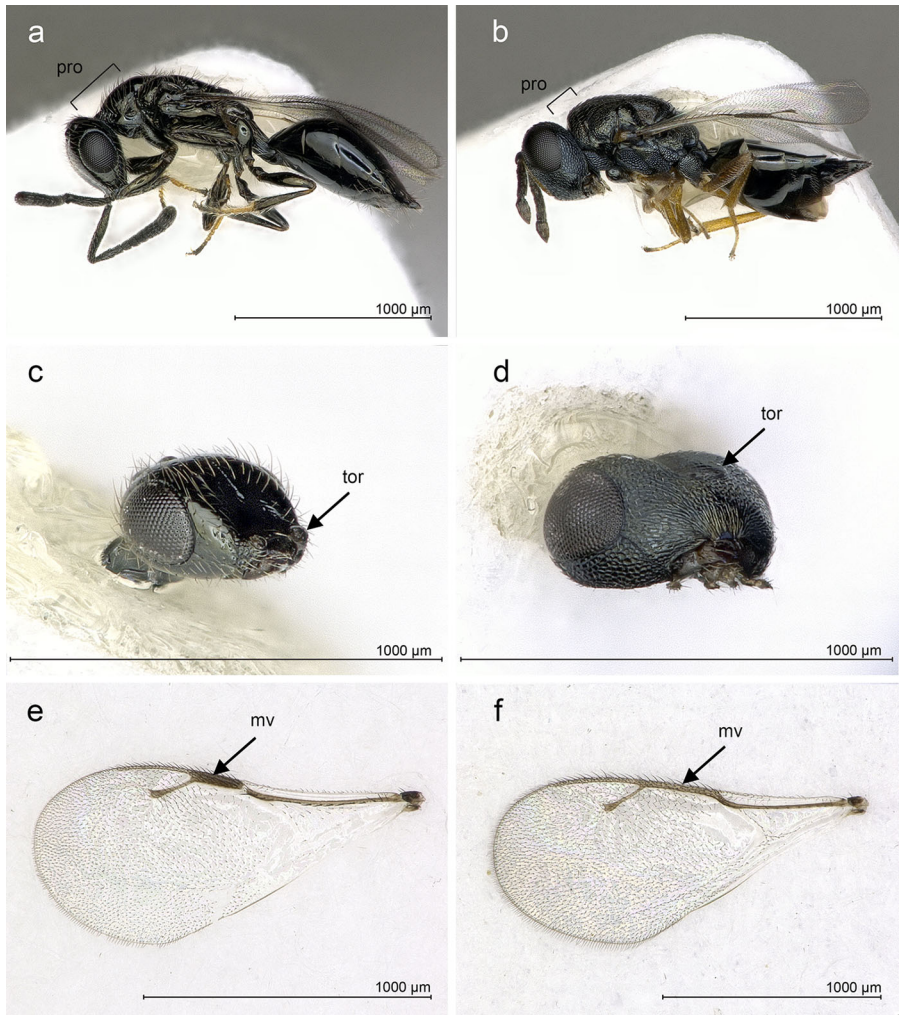

Fig. 2 Photographs of **a**, **c** *Spalangia erythromera* female; **b**, **d**, **e** *Pachycrepoideus vindemmiae* female; and **f** *Vrestovia fidenas* female. **a**, **b** body lateral (pro = pronotum); **c**, **d** head (tor = torulus); **e**, **f**, fore wing venation (mv = marginal vein)

4. Smaller, mesosoma lateral view strongly arched (Fig. 3c, d). Speculum of fore wing open below (Fig. 3f), pronotum collar with indistinct reticulation (Fig. 4f), malar depression with distinct reticulation (Fig. 4d)……..…***Vrestovia brevior*** Larger, mesosoma lateral view moderately arched (Fig. 3a/b), speculum of fore wings closed below by distinct setal line (Fig. 3e), pronotum collar longer with smooth hind margin and pronounced reticulation (Fig. 4e), malar depression with indistinct reticulation (Fig. 4c)……***Vrestovia fidenas***

Diagnosis

Vrestovia fidenas: body greenish and mesosoma lateral view only moderately arched (Fig. 3a, b). Antennae in both sexes with two small anelli (Fig. 4a). Malar depression with indistinct reticulation (Fig. 4c), pronotum collar relatively long with obvious shoulders and distinct reticulation between smooth stripe along hind margin and carina (Fig. 4e). Speculum of fore wings closed with distinct setal line (Fig. 3e). The distribution of *V. fidenas* is Europe and the Caucasus (Noyes 2018). Known hosts are *Drosophila phalerata* Meigen and *D. melanogaster* (Knoll et al. 2017; Offenberger and Klarenberg 1997).

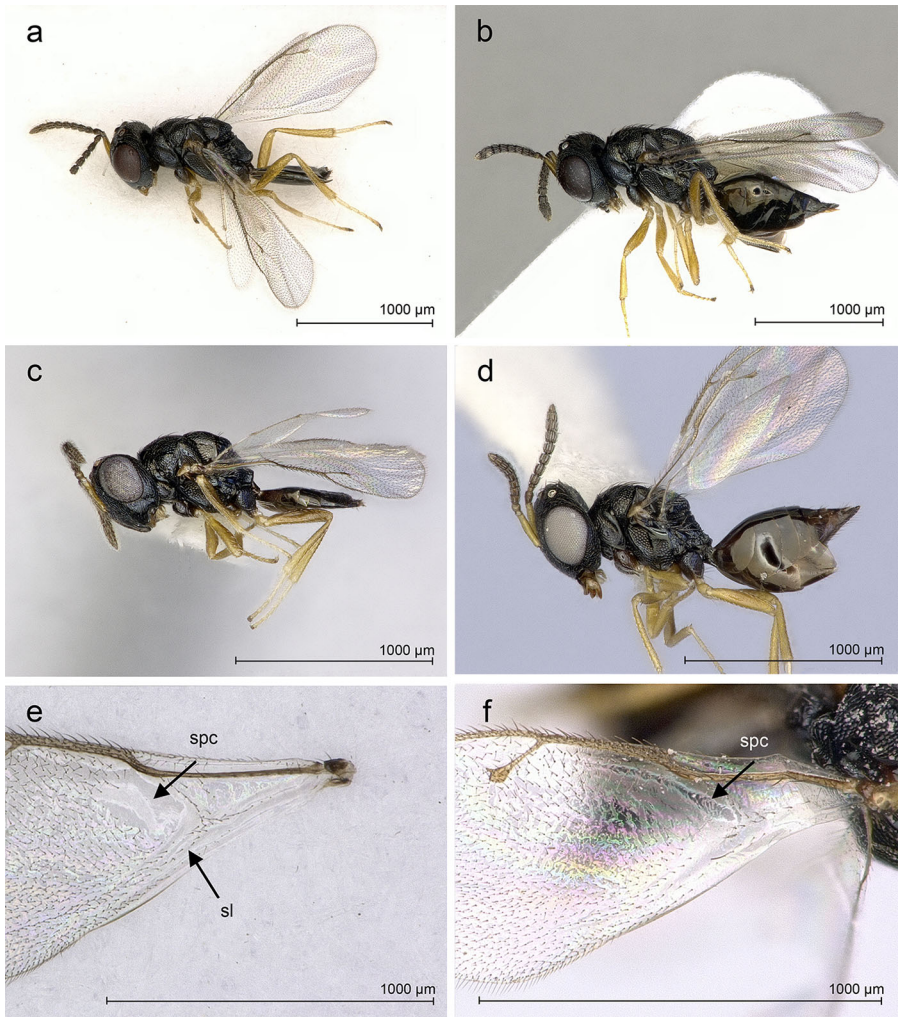

Fig. 3 Photographs of **a**, **b**, **e** *Vrestovia fidenas*; **c**, **d**, **f** *V. brevior*. **a**–**d** body lateral; **e**, **f** fore wing (spc = speculum, sl = setal line). **a**, **c** male; **b**, **d** female

Vrestovia brevior: body blackish and mesosoma lateral view strongly arched (Fig. 3c, d). Females easily distinguishable by having three anelli, the third anellus twice as long as the others (Fig. 4b). Malar depression with distinct reticulation (Fig. 4d). Pronotum collar is short with rounded shoulders, reticulation not pronounced (Fig. 4f). The distribution of *V. brevior* is North America and Europe (Germany, Italy, Switzerland) (Noyes 2018 and this study). Known hosts are *Drosophila* spp., *D. phalerata*, *D. melanogaster, D. subobscura*, and *Drosophila hydei* Sturtevant [Bouček 1993; Cara et al. (supplementary data S1, personal communication); Gillespie and colleagues (supplementary data S1); Offenberger and Klarenberg 1997; Thistlewood et al. 2013].

Discussion

In the present study, we investigated the biology of the parasitic wasp *V. fidenas*, a potential antagonist of the invasive *D. suzukii*. The results showed that *V. fidenas* is capable of parasitizing *D. suzukii* and can be considered a potential agent for the biological control of this vinegar fly. In addition, we document that *V. brevior*, another species of *Vrestovia* capable of

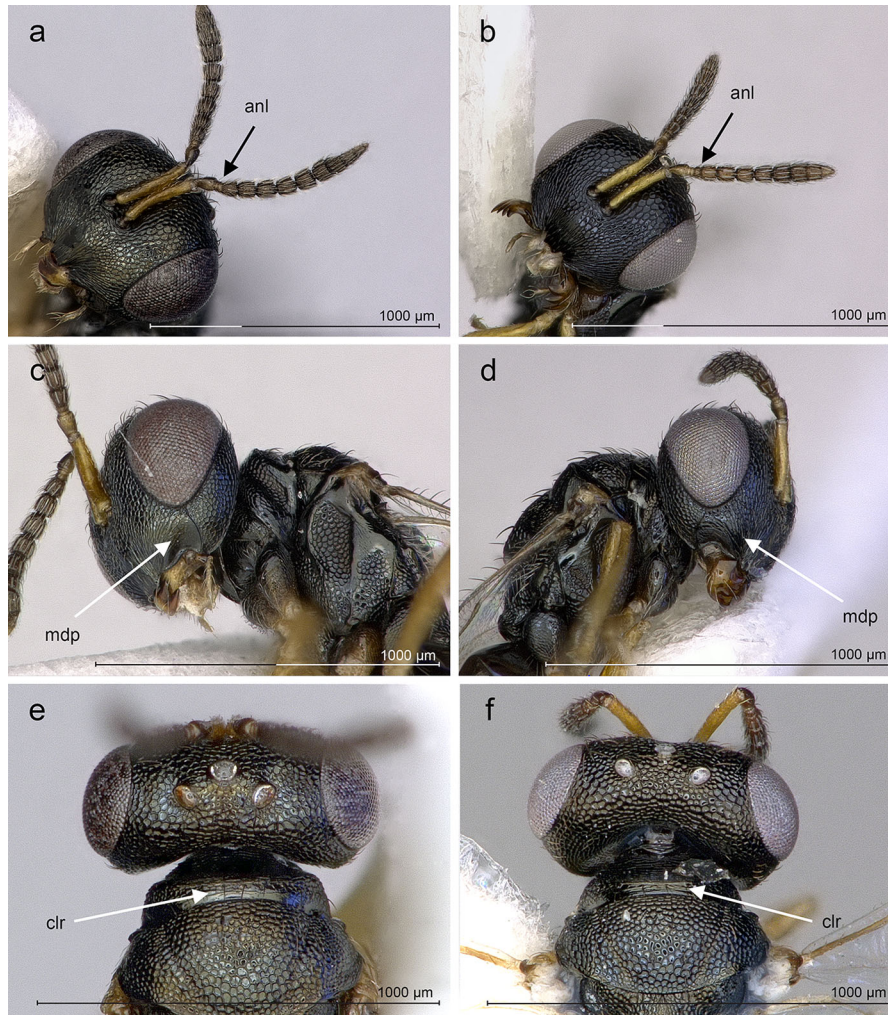

Fig. 4 Photographs of **a**, **c** and **e** *Vrestovia fidenas* female; **b**, **d** and **f** *V. brevior* female. **a** and **b** left antenna with anelli (anl); **c**, **d** malar space with malar depression (mdp); **e**, **f** pronotum collar (clr) dorsal

parasitizing *D. suzukii* and previously known only from North America, is also present in Europe.

Life-table parameters of *V. fidenas*

Females of *V. fidenas* produced significantly more offspring on pupae of *D. suzukii* than on pupae of *D. subobscura* (87 vs. 34 offspring per female). In addition, developmental time was shorter and offspring fitness (as indicated by adult weight) was higher on *D. suzukii* than on *D. subobscura.* The preferred host(s) of *V. fidenas* is still unknown, and *D. subobscura* was chosen as a surrogate native host, because it is a widespread frugivorous species that is found in similar habitats as *D. suzukii* and that can be reared under the same conditions.

The observed difference in the number of offspring produced per female was unlikely to have resulted from a difference in the health status of host pupae because emergence in the absence of the parasitoid was about 90% for both *Drosophila* species. In our rearing of these two species, development from egg to pupa takes about two days longer for *D. subobscura* than for *D. suzukii* under the same environmental conditions. Therefore, the pace of morphological and physiological changes within pupae likely differed between species. Preliminary observations indicate that pupae are most suitable for *V. fidenas* oviposition (in terms of female fecundity) when they are $\leq$ 24 h

old in the case of *D. subobscura* but $\leq$ 48 h old in the case of *D. suzukii* (unpublished data). In the life-table experiments, we used pupae that were $\leq$ 24 h old, and therefore the pupal stage of both hosts should have been optimal for parasitization.

The greater fecundity of *V. fidenas* on *D. suzukii* than on *D. subobscura* might also be explained by a difference in host defences against parasitoid attack. The native *D. subobscura* may have developed mechanisms that protect it against the native pupal parasitoid, but this would not be the case for *D. suzukii*, which, unlike *D. subobscura*, did not co-evolve with *V. fidenas*. To our knowledge, defence mechanisms by pupae of *Drosophila* have not been studied, but immune responses have been documented in lepidopterans (Freitak et al. 2003; Meylaers et al. 2007). Interestingly, larvae of *D. subobscura* (and other species of the obscura group) lack the ability to encapsulate parasitoid eggs, an ability that most *Drosophila* species have (Eslin and Doury 2006; Havard et al. 2009). It is possible that *D. subobscura* pupae can protect themselves against parasitism in a different way, e.g., by having a hard puparium or compounds unfavourable for parasitoid development. Because *Vrestovia* spp. have been reported in China and South Korea (Kamijo 1983; Yang 1996), co-evolution between *D. suzukii* and *Vrestovia* spp. cannot be ruled out. Still, *D. suzukii* does not appear to have developed defence mechanisms against these pupal parasitoids.

Both female and male *V. fidenas* had a shorter longevity when offered *D. suzukii* pupae rather than *D. subobscura* pupae for parasitization. For females, this is likely due to a trade-off between fecundity and longevity: parasitoid females might reabsorb their maturing eggs if conditions are not favourable, and use the gained energy to prolong their lives (Quicke 1997). This trade-off might explain the higher number of offspring and the shorter lifespan of *V. fidenas* females when parasitizing *D. suzukii* pupae rather than *D. subobscura* pupae. For males, on the other hand, the shorter lifespan when offered *D. suzukii* pupae rather than *D. subobscura* pupae might reflect a trade-off between longevity and the number of matings. Because the females produced more offspring on *D. suzukii*, they and the males they were paired with may have mated more often on *D. suzukii*, and perhaps male longevity is inversely related to the number of matings. Mating reduced the longevity of males of the pteromalid parasitic wasp *Nasonia vitripennis* (Walker) (Burton-Chellew et al. 2007) and also of both males and females of *Pteromalus cerealellae* (Ashmead) (Onagbola et al. 2007). In our study, males without access to females lived significantly longer than those with access, suggesting that mating reduced longevity.

Developmental time of *V. fidenas* was shorter on *D. suzukii* pupae than on *D. subobscura* pupae, except for the first cohort where the parasitoid batch used or irregularities of the climate chamber may have been responsible for the difference. Developmental time in parasitoids is often positively correlated with host mass (Harvey 2005), although the growth rate in large hosts can be increased, resulting in a higher gain in size during the same developmental time, particularly in idiobiont wasps, where host resources are limited. Consequently, such parasitoids ideally grow at the maximum possible rate for the given host quality (Mackauer et al. 1997). Because the pupae of *D. suzukii* weighed more than those of *D. subobscura* and because *V. fidenas* larvae developed faster when feeding on the heavier *D. suzukii* pupae, the *D. suzukii* pupae probably represented a better food resource than the *D. subobscura* pupae. Developmental time of the parasitoids might also be linked to the developmental time of their host. That *D. suzukii* develops faster than *D. subobscura* (unpublished data) might therefore explain the difference in *V. fidenas* developmental time on the two hosts.

A proxy for host size is its mass (Rogers et al. 1976), and heavier hosts offer more nutrients than lighter ones, which should affect offspring fitness (Honěk 1993). Pupae of *D. suzukii* were heavier than those of *D. subobscura*. Accordingly, the parasitoids that emerged from *D. suzukii* pupae were heavier than those that emerged from *D. subobscura*.

Life history strategy of *V. fidenas*

The dissections of parasitized *D. suzukii* pupae revealed that *V. fidenas* is an ecto-parasitoid. Its oviposition pattern indicates synovigeny, a term that describes that at least a few eggs are immature at female emergence, as opposed to pro-ovigeny, where all eggs are mature (Jervis et al. 2001). Adults of *V. fidenas* have also been observed to host feed (Collatz et al. 2019). All of these characteristics are associated with an idiobiont life history strategy in which host

development is stopped after initial parasitization (Quicke 1997). Because only one egg or larva was found per pupa and because the number of *V. fidenas* offspring never exceeded the number of pupae offered (Fig. 1b), *V. fidenas* is most likely a solitary parasitoid, producing only one offspring per host.

Comparison of *V. fidenas* and other pupal parasitoids of *D. suzukii*

Other pupal parasitoids of *Drosophila* that are capable of parasitizing *D. suzukii* include *P. vindemmiae*, *T. drosophilae*, and *Spalangia erythromera* Förster (e.g. Knoll et al. 2017; Mazzetto et al. 2016; Wang et al. 2016a, b). On *D. suzukii* pupae, lifetime fecundity was 68.4 offspring per *P. vindemmiae* female (Rossi Stacconi et al. 2015), and 52–64 per *T. drosophilae* female (Wang et al. 2016a; Zhu et al. 2017). In a bioassay in which these two parasitoids and *V. fidenas* were exposed to their hosts for five days, *V. fidenas* appeared inferior to the two other species (Knoll et al. 2017). It is likely that the observed lower efficacy was due to a lower initial egg load for *V. fidenas* compared to *T. drosophilae*, which emerges with a relatively high egg load (Wang et al. 2016a), and to the narrower range of host age suitability (0–48 h) of *V. fidenas* compared to *P. vindemmiae* (Rossi Stacconi et al. 2015). In the current study, the lifetime fecundity of *V. fidenas* on *D. suzukii* was 85.6 offspring per female, which was higher than previously reported for *P. vindemmiae* and *T. drosophilae*.

Developmental time of *V. fidenas* females in the current study was approximately 21 days on *D. suzukii* at 22 °C. This was longer than what has been reported for females of *P. vindemmia* and *T. drosophilae* (18 and 18–19 days, respectively) on the same host, but those previous experiments were carried out at warmer temperatures of 25 °C and 23–25 °C (Gabarra et al. 2015; Wang et al. 2016a). In our laboratory at 22 °C and with *D. melanogaster* as the host, developmental time is about 1–3 days shorter for *V. fidenas* than for *P. vindemmia* and *T. drosophilae* (unpublished data).

These parasitoid species also differ in other biological characteristics that must be considered before they are assessed or used as control agents against *D. suzukii*. *Pachycrepoideus vindemmiae* is a generalist species that parasitizes the pupae of many dipterans and also acts as hyperparasitoid (Noyes 2018). *Spalangia erythromera* is also a generalist species that attacks species in several dipteran families (Noyes 2018). Although *V. fidenas* has to date been associated with *Drosophila* species only, additional hosts may exist because field records are few and no host range testing has been conducted (Knoll et al. 2017; Offenberger and Klarenberg 1997). Different host ranges imply different host searching behaviours. Hence, the combined use of several parasitoid species might increase control of *D. suzukii*.

Trichopria drosophilae females emerge with a high mature egg load, can begin laying eggs on the first day, and they can lay a high number of eggs within a short time (Kaçar et al. 2017; Wang et al. 2016a; Zhu et al. 2017). Most females of *P. vindemmiae* and *V. fidenas*, on the other hand, have a pre-oviposition period of at least one day before they begin laying eggs and can continue to lay eggs over a long period (Rossi Stacconi et al. 2017, this study). At the beginning of a *D. suzukii* infestation, *T. drosophilae* could be released to rapidly parasitize the already developed *D. suzukii* pupae. *Vrestovia fidenas* and *P. vindemmiae*, on the other hand, could contribute to the continuous control of later developing pupae. *Pachycrepoideus vindemmiae* and *T. drosophilae* are frequently collected with sentinel traps, and conservation measures might therefore increase the effects of these rather common parasitoids. *Vrestovia fidenas* has only been collected in a few places and at low numbers, and whether its effects on *D. suzukii* could be increased by augmentative releases remains to be investigated. In summary, all of these species could play a role in *D. suzukii* control, either alone or together.

Vrestovia brevior: new record for Europe

A second species of *Vrestovia* could be interesting in this context: *V. brevior*, another parasitoid of *Drosophila* described for North America, is also known to parasitize and develop in *D. suzukii* (Thistlewood et al. 2013). It was collected in low numbers from *Drosophila* spp. (probably also *D. suzukii*) in Canada and the USA and was successfully reared on *D. suzukii* pupae (Thistlewood et al. 2013). Its life table parameters are not known on *D. suzukii* or on any other host. We document here that *V. brevior* is also present in Europe, where it has previously been misidentified as *V. fidenas* (see supplementary data S1). A comparison of *Vrestovia* individuals from museums as well as some individuals collected from Monteggio (Ticino,

Switzerland) in 2017 (Cara et al., personal com.) with *V. fidenas* from our cultures revealed differences between the species in the number of anelli as well as in the forewing pilosity. Some of the examined individuals had characteristics that matched those of *V. fidenas*, but others had characteristics that matched those described for *V. brevior*. This proves that both species occur in Europe. Therefore, it is important to correctly distinguish between the two species, in particular when they are evaluated as potential biological control agents of *D. suzukii*.

The photographs provided in our publication can be used by non-taxonomists to distinguish the most common pteromalids of *Drosophila* including the two *Vrestovia* species. For verification, specimens of potential *Vrestovia* spp. should be sent to a taxonomist.

Acknowledgements This research project was funded by the *Drosophila suzukii* R & G Task Force (funded by the Swiss Federal Office for Agriculture FOAG). We thank Gary Gibson of the Canadian National Collection of Insects for loaning us specimens of *Vrestovia brevior*. We also thank Valeria Trivellone, Corrado Cara and Michela Meier for giving their specimens of *Vrestovia brevior* they collected in 2017. And thanks to Jörg Romeis and three anonymus reviewers for their valuable comments on earlier versions of this manuscript.

Compliance with ethical standards

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

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