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Mantidfly larvae use cues on substrate to locate and distinguish different sexes and life stages of potential spider hosts

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In many animals, early-life decisions influence long-term fitness. Mantidflies are spider egg predators; their tiny larvae climb aboard spiders to find eggs, but little is known about how they find spiders. We tested the hypothesis that mantidfly larvae (*Dicromantispa sayi*) detect and respond to substrate-borne spider cues (e.g., silk and/or excreta). We presented larvae with filter paper exposed to different types of spiders (adult female, adult male, or juvenile *Habronattus trimaculatus* jumping spiders) versus a no-cue control. Larvae spent more time on filter paper with spider cues. We then tested the hypothesis that mantidflies make finer distinctions between spiders when given direct choices between these cues. Larvae did not discriminate between sexes but spent more time (and exhibited more phoretic behavior) on filter paper with female or male cues compared with juvenile cues. While this suggests that mantidflies actively seek out adult spiders, we also found that adult spiders were more active than juveniles and may have simply deposited more silk and excreta, providing a stronger cue to detect. We discuss these findings in the context of the risks and benefits of different spider hosts, and how early-life spider-searching strategies may shape a mantidfly's long-term fitness.

Early life decision-making strategies that influence habitat or resource quality during development can affect future fitness and therefore should be under strong selection (e.g., insects[1–3], birds[4]). These decisions might be especially crucial for species without parental care, where free-living juveniles must begin to forage on their own immediately after hatching. For organisms that rely on other animals as hosts or to transport them to resources (e.g., phoresy of arthropods and nematodes)[5], these decisions become even more critical. In many animals, juveniles make selective choices of hosts using criteria like host size, life stage, or sex, that are presumably linked to host quality (e.g., mites[6], flatworms[7], flies[8]). The ability of juveniles to make good foraging decisions early on may greatly influence long-term fitness (e.g., flies[9], birds, reptiles, amphibians, arthropods[10]).

However, there are also circumstances where we might expect animals to be less selective in their decision-making. For example, theory predicts that species with high mortality rates and short life spans should not show strong preferences for specific hosts or foraging patches over others (e.g., theoretical models[11], aphids[12]). Additionally, heightened predation risk and increased competition should also decrease an animal's choosiness while foraging (e.g., beetles[13]).

Collectively, the above theory has helped us make sense of the diversity of early-life decision-making strategies of many animals (fishes[14], amphibians, reptiles, mammals, birds, invertebrates[15]). However, some animals possess unique combinations of the above traits, which make it harder to generate clear predictions from existing theory. These animals, especially those belonging to understudied taxa, have the potential to offer fresh insights for the field.

The mantidflies (Neuroptera: Mantispidae) are understudied spider egg predators (Fig. 1) that offer a unique opportunity to explore the costs and benefits of making (or not making) active host-seeking choices as juveniles. While adult mantidflies are generalist predators that superficially resemble small praying mantises (despite being in a different taxonomic order than mantises) (Fig. 1B), it is the larvae that seek out spider eggs (Fig. 1A). These larvae use two main strategies for accessing eggs: spider boarding and egg sac locating[16,17]. Spider boarding occurs when larvae perform phoretic behavior to contact and climb onto live spiders and enter egg sacs while they are constructed by the female spider[16,17]. Phoretic behavior in mantidfly larvae consists of

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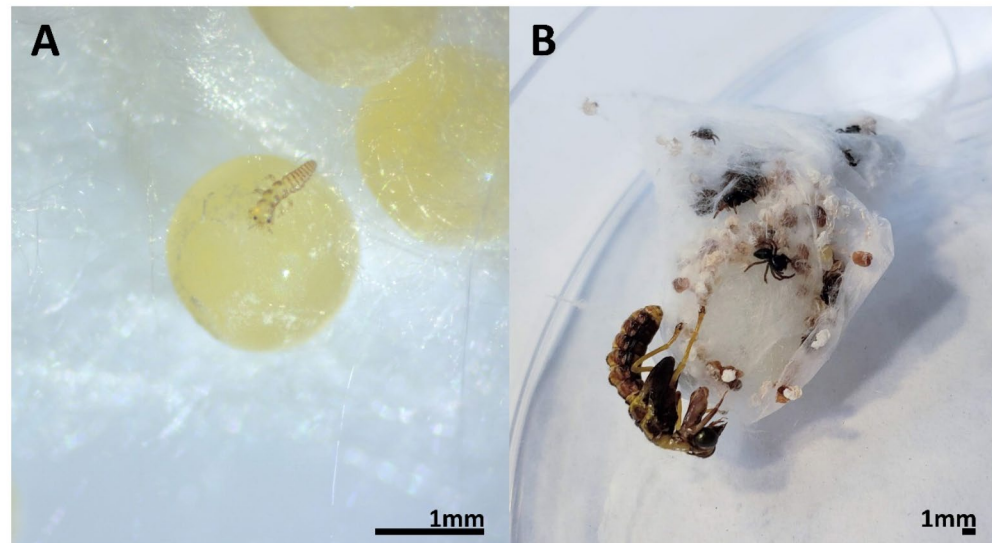
Fig. 1. (**A**) First instar mantidfly larva (*Dicromantispa sayi)* investigating silk and spider eggs from the jumping spider, *Habronattus trimaculatus*. (**B**) Pharate adult mantidfly (*Dicromantispa sayi*) emerged from a jumping spider, *Phidippus regius*, egg sac before molting a final time to the adult stage.

the larva raising its entire body vertically into the air while balancing on caudal suckers, and swaying from side to side[16](Supplementary Video 1). First instar mantidfly larvae periodically perform phoretic behavior while moving along the substrate and actively searching for spider hosts. Phoretic behavior is mainly used for dispersal in many arthropods (ticks, mites, parasitoid wasps, pseudoscorpions, etc.) to aid in making contact with a host organism[5]. In mantidflies, egg sac locating occurs when larvae actively search for and enter egg sacs that have already been constructed[16,17]. Some species use either one strategy or the other, while other species have the ability to do both[16,17]. However, regardless of their strategy for finding eggs, once they locate an egg sac and begin feeding, larvae are limited to the finite resources within that single egg sac[16–18]. Previous work has shown that the size of the spider egg sac strongly affects a mantidfly's final adult body size[18,19], suggesting that the initial decision to enter a particular egg sac, to board a particular spider, or even to forgo an opportunity and continue searching may be particularly important. Factors affecting mating behavior and reproductive success in mantidflies is largely unknown, but body size likely influences the risk of aggression and sexual cannibalism in this system[16,18,19]. As such, host choices made by juveniles are likely to substantially influence their adult body size and subsequently their reproductive success.

Aside from the knowledge of the existence of the two strategies for accessing eggs described above, little is known about how mantidfly larvae search for hosts or whether they make any distinctions between different spiders or different egg sacs. First instar larvae hatch from egg clutches that can contain thousands of siblings that all hatch within 24 h of one another[18]. They are extremely small (approximately 1 mm in total body length; Fig. 1A) and cannot survive long without finding a spider host or spider egg sac[20,21]. First instar larvae have been observed attached to a wide variety of spider species and spider life stages (adult males, adult females, and immatures)[22,23]. They can move from one spider to another during spider mating and cannibalism and they can remain on a single juvenile spider through the molting process[16,18,24]. They can even maintenance feed on spider hemolymph when they do not move into a spider egg sac right away[25].

With such a wide range of possible spider hosts and trajectories for their future development, are mantidfly larvae using fine distinctions to locate the ideal spider hosts and eggs? Are these distinctions associated with ultimately ending up in the largest possible egg sac that would maximize their fitness? Or are they simply boarding any spider they encounter to increase their chances of finding an egg sac at all, and/or to disperse quickly from siblings and reduce competition? Being too choosy might result in never finding a suitable egg sac, but a lack of choosiness could result in a larva boarding a spider that will not immediately deliver them to eggs (e.g., an older female spider that has already laid eggs and will not lay any more or a small juvenile spider that will not lead them to eggs until after they mature).

Here, we use the mantidfly species, *Dicromantispa sayi* (Banks, 1897) (synonyms: *Dicromantispa fuscicornis*, *Mantispa uhleri*, *Mantispa fuscicornis*, *Mantispa sayi*), to investigate questions about early life decisions in spider egg locating and host selection. In this species, larvae can access eggs either by locating them directly or by boarding spiders[18]. First, we tested the hypothesis that first instar mantidfly larvae can distinguish between the presence and absence of substrate-borne spider cues by giving larvae a choice test between filter paper with spider cues (i.e., silk and excreta) and filter paper with no cues. Here, we use the term cue to describe any stimuli that are present in the environment that might influence a mantidfly larva's behavior. In these tests, we used spider cues from adult female, adult male, and juvenile spiders. Because mantidfly larvae are found on all

of these stages of spiders in the field[18,23], we predicted that they would show a preference for filter paper with spider cues (over filter paper with no cues), regardless of the sex or life stage of the spider. Second, we tested the hypothesis that mantidfly larvae can make finer distinctions between cues from spider hosts when given a direct choice between different spider sexes and life stages. We predicted that larvae would perform more phoretic behavior and spend more time in the presence of cues from adult female spiders compared to adult male or juvenile spiders because females may offer the most direct pathway to egg sacs. However, we also considered the possibility that mantidfly larvae would prefer adult male spiders over juveniles because males actively seek out receptive females with which to mate, and mating is often followed by the production of eggs. As such, male spiders may be able to deliver mantidfly larvae to females at precisely the time when those females are getting ready to lay eggs. Finally, we predicted that cues from juvenile spiders would be the least attractive to mantidfly larvae because juvenile spiders are unlikely to lead immediately to eggs. While a mantidfly larva can survive on a juvenile spider until it matures[18], there are likely to be increased risks and costs, such as the possibility of the spider being eaten by a predator and the larva needing to navigate multiple spider molting events. The unique biology and natural history of mantidflies may provide intriguing insights into how early life decisions can shape long-term fitness.

Methods
Collection and maintenance
We collected adult *D. sayi* females ($n = 18$) using a blacklight from May through September 2019 and 2021 in Gainesville, Florida, USA. We housed females individually in clear plastic boxes ($10.16 \times 10.16 \times 12.86$ cm) with a mesh covering on the top for ventilation (5.5 cm diameter) and we fed them approximately 10 fruit flies (*Drosophila melanogaster*) daily. We provided each with a moistened organic cotton ball and sprayed their boxes with water daily to prevent desiccation.

Spider cues for these experiments were sourced from *Habronattus trimaculatus* jumping spiders (family Salticidae) because jumping spiders are common hosts for mantidflies and have been used in previous studies on mantidflies[18,22,23,25]. We collected spiders via sweep net from May through September 2019 and 2021 in Gainesville, Florida, USA ($n = 60$ adult male, $n = 60$ adult female, $n = 60$ juveniles; juveniles were less than or equal to 2 mm in total body length). Because they were collected as adults, the mating status of the males and females was unknown. We housed spiders individually in clear plastic boxes ($10.16 \times 10.16 \times 12.86$ cm) with a mesh hole for ventilation (5.5 cm diameter), fed them a diet of pinhead crickets (*Gryllodes sigillatus*) approximately equal to their body weight three times per week, and sprayed their houses with water daily.

Experiment 1: Are Dicromantispa sayi larvae attracted to spider cues?
To test whether mantidfly larvae can detect and are attracted to spider cues compared to a no cue control, adult female mantidflies ($n = 7$) that mated in the field prior to collection were allowed to lay clutches of eggs in the lab. Mantidflies can lay multiple clutches throughout their lifetime, each with hundreds to even thousands of eggs[16]. After laying an egg clutch (on the plastic walls of their home box), females were removed from the box and re-housed and the eggs were monitored daily until hatching was observed ($n = 21$ egg clutches). All larvae within a clutch emerge simultaneously and gather underneath the egg stalks before dispersing (Lietzenmayer, pers. obs.). When this gathering was observed for each individual egg clutch, three different *Habronattus trimaculatus* spiders (adult female, adult male, and juvenile) were individually placed in separate 9 cm petri dishes lined with 9 cm round filter paper (Fisherbrand, Fisher Scientific, Waltham, USA) where they were given 24 h to lay silk, excreta, and other potential olfactory or tactile cues onto the filter paper (following methods used in several previous spider studies[26,27]). We cleaned petri dishes with 95% ethanol and allowed them to dry fully prior to trial setup to ensure that no other olfactory or tactile cues were present.

On the following day, testing arenas were created using 9 cm petri dish lids. On one side of each arena, we placed a half of a piece of filter paper with cues from a spider (either an adult female, adult male, or juvenile) while the other side of the arena had a filter paper half with no spider cues present (i.e., a control piece of filter paper that had not been exposed to any spiders) (Fig. 2). We secured the filter paper to the arena using a small amount of Elmer's glue along the perimeter of each filter paper half to ensure that mantidfly larvae could not crawl underneath. We ensured the glue was fully dry before the start of each trial and used approximately equal amounts across all locations of the arena for all treatments (to ensure that any effect of the glue did not introduce biases in our experiments). The side of the arena with spider cues was chosen randomly with the flip of a coin in case larvae had any biases for one side over the other. Coin-flipping did not result in a perfect 50/50 distribution; spider cues were positioned on the left side in 50% of trials with female cues, 47% with male cues, and 41% with juvenile cues. A neutral zone approximately 1 mm in width separated the two halves of filter paper (Fig. 2). The top edge of the arena was lined with a small Vaseline barrier (~ 1 mm thick) to ensure the larvae stayed inside the arena. A single first instar larva (Fig. 1A) was then transferred using a microbrush to the middle of the neutral zone of the arena and given two minutes to explore the arena before observation began.

To see if mantidfly larvae are attracted to spider cues (in the absence of a spider), we observed the larvae for 90 min in real time and recorded the total time they spent on each side of the arena ($n = 34$ larvae per spider type, 102 larvae total). We opted for direct observation of trials instead of video recording as mantidfly larvae are extremely small and difficult to see on video playback. While we did not quantify the side of the arena the larvae first entered after placement in the neutral zone during the acclimation period, we did record the side of the arena that the larvae were on at the start of the 90-minute observation period; this was done post-hoc to explore the idea that the larvae were using olfactory cues to determine which side of the arena to explore first.

Each spider and mantidfly larva were only used once, and mantidfly larvae from the same egg clutch were equally distributed across all treatments. Larvae came from the clutches of seven different females and no more than 10 individual larvae from a single maternal female were used in each treatment.
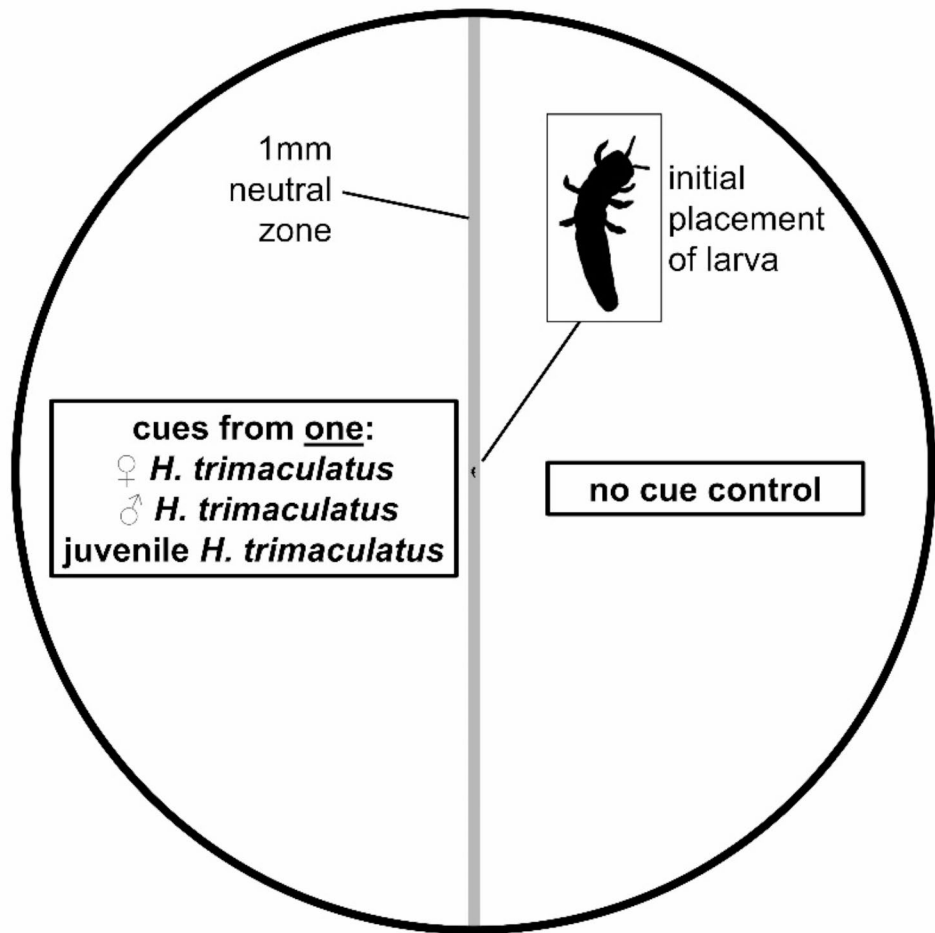
Fig. 2. Schematic of the arena used in Experiment 1 (illustration is drawn to scale). One side included filter paper with spider cues that originated from one of three treatments (adult female, adult male, or juvenile) and the other side was a clean filter paper half with no spider cues. The side of the arena (left or right) to be assigned spider cues (vs. the no cue control) was randomized for each trial.

Statistical analyses for Experiment 1. To test whether spider cues overall were detected by mantidfly larvae, we ran a linear mixed-effects model using pooled data from all spider types with arena side (spider cues or no cue control) as the main factor, mantidfly maternal ID and trial ID as random factors, and total time spent on the filter paper as the response variable. Maternal ID was included to account for variation among mantidfly egg clutches from different mothers. Trial ID was included because spider cues and a no-cue control were paired within each trial.

In follow-up analyses, the data for each spider sex/age category (adult female, adult male, and juvenile) were analyzed separately. This allowed us to assess whether larvae responded to cues from each of the spider types (regardless of sex or life stage). We ran linear mixed-effects models for each spider type separately using the same model described above.

All of the models described above indicated singularity, with both random effect variances (maternal ID and trial ID) estimated as 0. Because neither of our random effects were explaining any variation in the data, models with and without the inclusion of the random effects show identical statistical results. See Supplementary Table 1 for the comparison of models with and without random effects.

To make initial comparisons of larval attraction to different spider sexes or ages, we subsetted the data to only include time spent on the spider cue side of the arena (excluding the data from the no cue control side of the arena). We then compared the time spent on the cues from the three different spider sexes/ages using a linear mixed-effects model with spider type (adult female, adult male, juvenile) as the main factor, maternal ID as a random factor, and total time spent on the filter paper as the response variable. Trial ID did not need to be included in this model because we did not include data from the no cue control side of the arena. We used Tukey adjustment for multiple comparisons to determine pairwise differences among the three spider types included in the model.

To explore the idea that mantidfly larvae use olfactory cues to make initial decisions on which side of the arena to search first, we ran one-sample proportion tests comparing the proportion of larva that started on the side of the arena with spider cues (females, males, or juveniles) to the null hypothesis of a 0.5 proportion. We ran each test without the Yates continuity correction because all expected counts were greater than 5.

All linear mixed-effects models were made with the lmer() function in the R package lme4[28]. All linear models were performed with the lm() function. All linear models and linear mixed-effects models had Gaussian distributions. Tukey adjustment for multiple comparisons and confidence intervals were calculated using the emmeans function in the emmeans package[29]. P-values were calculated using the Anova() function in the car package[30] for lm() functions and the anova() function for lmer() functions. Linear model assumptions of normality of residuals and homogeneity of variance were confirmed by visual assessment of histograms and qqPlots of residuals. Data were analyzed in RStudio using R version 4.3.1[31]. All raw data and R code are available on Dryad at: https://doi.org/10.5061/dryad.kd51c5bft.

Experiment 2: Do Dicromantispa sayi larvae distinguish between cues from different spider sexes and life stages?
In light of our findings from Experiment 1 suggesting that the mantidfly larvae respond to the presence of spider cues (vs. a no-cue control, see Results), we went on to conduct Experiment 2. The goal of Experiment 2 was to determine if mantidfly larvae discriminate between different types of spider cues when presented with them simultaneously. We prepared arenas following the same protocol described above, but instead of spider cues being paired with a no-cue control, we randomly assigned each mantidfly larvae to one of three preference test treatments for direct comparisons between the cues from different spider sexes and life stages (adult female vs. adult male ($n = 26$), adult female vs. juvenile ($n = 26$), and adult male vs. juvenile ($n = 26$)). We used a coin flip to determine the side of the arena designated to each cue type. Coin-flipping did not result in a perfect distribution for any spider cue combination: female cues were on the left side in 53% of trials when compared with male cues, and in 46% of trials when compared with juvenile cues. Male cues were on the left side in 60% of trials when compared with juvenile cues. As in Experiment 1, we recorded the total time larvae spent on each side of the arena within a 90-minute observation period.

We also added phoretic behavior as an additional response variable in Experiment 2. We would expect this behavior to occur more often when the larvae detect cues from a preferred host. To quantify phoretic behavior, we recorded the number of times that each larva raised its body vertically into the air while balancing on caudal suckers (as described in the Introduction, see Supplementary Video 1). In addition to direct counts of phoretic behavior, we also converted these counts to a rate by calculating the number of phoretic behaviors observed per minute on each side of the arena. Using two different metrics for phoretic behavior allowed us to determine whether mantidflies exhibit phoretic behavior at regular intervals while host searching (with counts being proportional to the time spent on each side of the arena) and whether they increase/decrease their frequency of phoretic behavior when in the presence of particular spider cues.

In Experiment 1, we noticed that the mantidfly larvae slowed down and directly made contact with spider silk that had been deposited on the filter paper. Because spiders lay down silk draglines as they move through their environment, more active spiders are likely to lay down more silk than less active individuals. If this is the case, sex- and age-related variation in activity among our spider groups may have affected how the mantidfly larvae responded to the treatment groups. To account for this variation, we recorded the activity level of each spider for 15 min after they were placed in the petri dish (during the spider cue collection phase of the experiment). A 2×2 cm grid was drawn on the lid of the petri dish using a ruler and fine tip pen, and the petri dish was videotaped directly overhead with a Sony Handycam (HDR-PJ540, Sony Electronics Inc., San Diego, USA). The total number of 2×2 cm boxes the spider crossed with their entire body in 15 min was recorded as an estimate of activity.

Statistical analyses for Experiment 2. To test directly whether larvae exhibit a heightened attraction to cues from certain spider sexes or life stages over others (when presented with them simultaneously), we ran a separate linear mixed-effects model for each choice-test combination of spider types (adult female vs. adult male, adult female vs. juvenile, adult male vs. juvenile). Each model included spider type as the main factor, maternal ID and trial ID as random factors, and total time spent as the response variable for the same reasons described for the models in Experiment 1. These models also indicated singularity and estimated that the random effect variances for maternal ID and trial ID were 0. We ran models with and without the inclusion of the random effects.

As in Experiment 1, we determined if mantidfly larvae use olfactory cues to make initial decisions on which side of the arena to search first by running exploratory one-sample proportion tests. In these tests for Experiment 2, we compared the proportion of larva that started on the side of the arena with female spider cues in tests where larvae had the choice between female vs. male or female vs. juvenile cues, and male spider cues in tests with male vs. juvenile cues, to the null hypothesis of a 0.5 proportion. We ran each test without the Yates continuity correction because all expected counts were greater than 5.

To investigate the effect of spider type on phoretic behavior, we ran two different linear mixed-effects models for each of the phoretic behavior metrics described above: generalized linear mixed-effects models with Poisson distribution with direct counts of phoretic behavior as the response variable and linear mixed-effect models with number of phoretic behaviors per minute as the response variable. Each model included spider type as the main factor (one model for each combination of spider type), maternal ID and trial ID as random factors, and phoretic behavior (either direct counts or rate) as the response variable. Because singularity was also present in these models, we ran models with and without random effects included.

To assess whether spider activity rates among different spider life stages and sexes were similar, we ran a linear model with spider type (adult female, adult male, juvenile) as the main factor and total boxes crossed in the 15-minute observation period as the response variable. Because our data did not meet the assumptions for a linear model, we also ran a nonparametric Kruskal-Wallis test and pairwise comparisons using Dunn tests with

Bonferroni correction. We also ran an exploratory linear mixed-effects model to ask whether spider activity alone (independent of spider sex/stage) predicted how much time a mantidfly larvae spent on each side of the arena, using spider activity as the main factor, total time spent by the mantidfly as the response variable, and the random factors maternal ID and trial ID. Due to singularity in the model, we also ran the same model without the two random factors.

All linear mixed-effects models and linear models were made using the same functions described for Experiment 1. For phoretic behavior rate models, data were log-transformed when model assumptions were not met. All generalized linear mixed-effects models were made with the glmer() function in the lme4 package and checked for overdispersion using the overdisp.glmer() function in the RVAideMemoire package[32]. Normality of residuals for generalized linear mixed-effects models was checked using the simulateResiduals() function in the DHARMa package[33]. Tukey adjustment for multiple comparisons and confidence intervals were calculated using the emmeans function in the emmeans package[29]. P-values for generalized linear models were calculated using the Anova() function in the car package[30]. Nonparametric tests used to compare spider activity levels in Experiment 2 were performed with the rstatix package[34] because assumptions were not met. Data were analyzed in RStudio using R version 4.3.1[31]. All raw data and R code are available on Dryad at: https://doi.org/10.5061/dryad.kd51c5bft.

Results

Experiment 1. When data for all spider types were pooled, we found that mantidfly larvae spent more time on filter paper with spider cues than on filter paper with no cues (Table 1, Supplementary Table 1).

When analyzing the data for each spider type separately, we found the same pattern for each spider type (Fig. 3; Table 1, Supplementary Table 1).

Although larvae in Experiment 1 were not presented with multiple cues simultaneously (as they were in Experiment 2), we found that larvae spent different amounts of time on filter paper from different spider types ($F_{(2,93.85)} = 4.52$, $p = 0.013$). Larvae spent more time on filter paper with cues from adult males compared to juveniles (t = -2.80, $p = 0.017$). There were no detectable differences between the amount of time spent on filter paper with cues from adult females and juveniles (t = 0.45, $p = 0.896$) or adult females and adult males (t = -2.35, $p = 0.054$). Larvae spent relatively little time in the neutral zone (all spider types pooled: 3.25 ∓ 3.25 min).

Larvae did not show any initial bias toward the side of the arena with spider cues in trials with female cues (proportion starting on female cue side = 0.52, $\chi 2 = 0.03$, df = 1, $p = 0.86$), male cues (proportion starting on male cue side = 0.42, $\chi 2 = 0.76$, df = 1, $p = 0.38$), nor juvenile cues (proportion starting on juvenile cue side = 0.39, $\chi 2 = 1.48$, df = 1, $p = 0.22$).

Experiment 2. In Experiment 2, direct comparisons between spider types showed that mantidfly larvae spent more time on filter paper from adult females compared to juvenile spiders (Fig. 4A; Table 2, Supplementary Table 2). Larvae also spent more time on filter paper from adult male compared to juvenile spiders (Fig. 4A; Table 2, Supplementary Table 2). We found no difference between filter paper from adult female and male spiders (Fig. 4A; Table 2, Supplementary Table 2). As in Experiment 1, larvae spent relatively little time in the neutral zone (all treatments pooled: 3.27 ∓ 2.36 min).

In our exploratory analyses, larvae showed no initial bias in the side of the arena that they moved to at the start of the trial in any choice tests (female vs. male: proportion starting on female cue side = 0.38, $\chi 2 = 1.50$, df = 1, $p = 0.22$; female vs. juvenile: proportion starting on female cue side = 0.38, $\chi 2 = 1.50$, df = 1, $p = 0.22$; male vs. juvenile: proportion starting on male cue side: 0.35, $\chi 2 = 2.13$, df = 1, $p = 0.14$).

Analyses investigating phoretic behavior in Experiment 2 follow similar patterns shown above for time spent on filter paper. For analyses using direct counts of phoretic behavior, we found that mantidfly larvae performed more phoretic behavior when in the presence of female cues compared to juvenile cues (Table 3, Supplementary

	Time spent mean ∓ se (min)	95% CI	F	df	p
Pooled data (n = 102)			379.19	1,202	**<0.0001**
Spider cues	61.7 ∓ 1.39	[58.7–64.7]			
No cues	25.0 ∓ 1.39	[22.1–28.0]			
Spider type					
Adult female (*n* = 34)			95.69	1,66	**<0.0001**
Spider cues	59.7 ∓ 2.49	[54.4–65.1]			
No cues	26.9 ∓ 2.49	[21.5–32.2]			
Adult male (*n* = 34)			261.01	1,66	**<0.0001**
Spider cues	67.1 ∓ 2.19	[62.4–71.8]			
No cues	19.5 ∓ 2.19	[14.8–24.2]			
Juvenile (*n* = 34)			89.13	1,66	**<0.0001**
Spider cues	58.3 ∓ 2.21	[53.9–62.8]			
No cues	28.8 ∓ 2.21	[24.4–33.2]			

Table 1. Results of Experiment 1 where *Dicromantispa sayi* larvae were given the choice between two halves of filter paper: one treated with spider cues and one that had no spider cues (control). Results shown are the amounts of time the mantidfly larvae spent on each half of the filter paper in minutes.
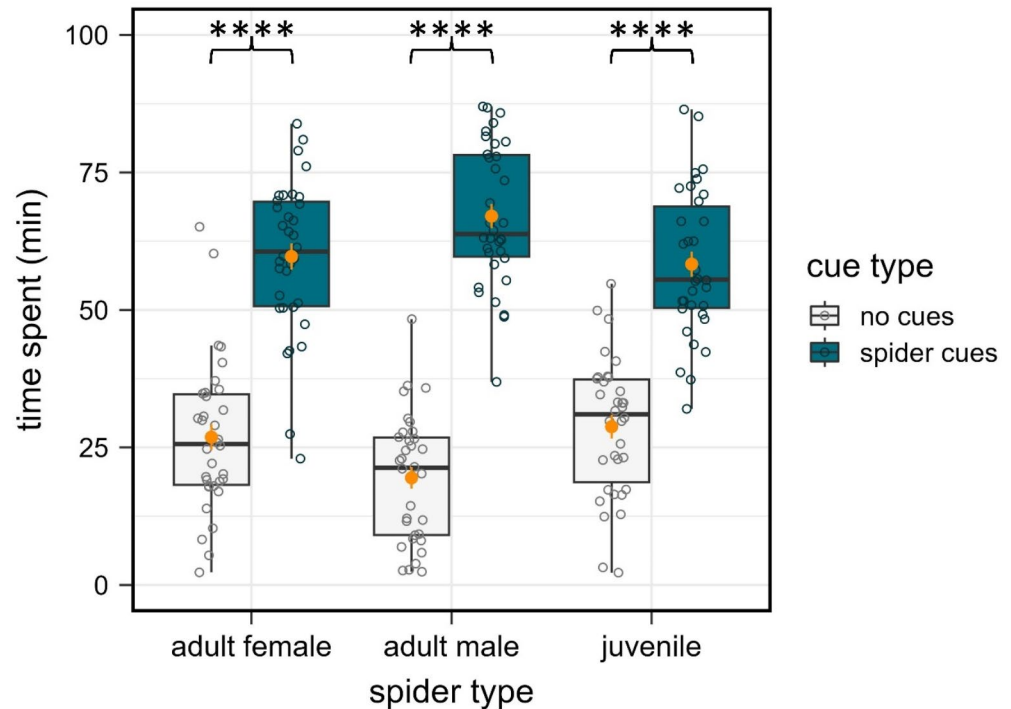
Fig. 3. Results of Experiment 1 where mantidfly larvae (*Dicromantispa sayi)* were given the choice between filter paper halves with and without spider cues. Plots indicate the amount of time the mantidfly larvae spent on each half of the filter paper during the 90-minute trial period, during tests with cues from adult female, adult male, or juvenile *Habronattus trimaculatus* spiders. Light orange points with error bars indicate mean and SE, respectively while boxplots show the median, upper and lower quartiles, and the maximum and minimum values. Open circles represent raw data. "****" indicates $p < 0.0001$.

Table 3) and male cues compared to juvenile cues (Table 3, Supplementary Table 3). We found no difference in phoretic behavior in mantidfly larvae between female and male cues (Table 3, Supplementary Table 3). Means and 95% confidence intervals for models using counts of phoretic behavior are presented in Table 3.

The rate of phoretic behavior per minute was also higher when in the presence of female cues compared to juvenile cues (adult female: 0.23∓0.03; CI [0.17,0.29]; juvenile: 0.11∓0.03; CI [0.04,0.17]; log-transformed: $F_{(1,25.22)} = 23.01$, $p < 0.0001$; Fig. 4B, Supplementary Table 4), and in the presence of male cues compared to juvenile cues (adult male: 0.25∓0.054; CI [0.12–0.37]; juvenile: 0.16∓0.05; CI [0.03–0.28]; log-transformed: $F_{(1,38.95)} = 18.83$, $p < 0.0001$; Fig. 4B, Supplementary Table 4). We found no difference in rate of phoretic behavior when in the presence of adult female or male cues (adult female: 0.25∓0.03; CI [0.18–0.32]; adult male: 0.19∓0.03; CI [0.12–0.26]; $F_{(1,25)} = 3.49$, $p = 0.074$; Fig. 4B, Supplementary Table 4).

We found that the different types of spiders (adult female, adult male, juvenile) had different activity levels during the spider cue collection period of Experiment 2 (Kruskal-Wallis χ2 = 11.0, $p = 0.004$, df = 2). Adult females (272.48∓34.01 boxes crossed) were more active than juveniles (137.19∓22.28 boxes crossed; Dunn test with Bonferroni correction, $p = 0.002$), and adult males (244.69∓30.98 boxes crossed) were more active than juveniles (Dunn test with Bonferroni correction, $p = 0.009$). Adult females and males showed similar activity rates (Dunn test with Bonferroni correction, $p = 0.640$). Overall, regardless of spider type, we found that mantidflies spent larger amounts of time on the side of the arena with cues from spiders that were more active ($F_{(1,76)} = 5.294$, $p = 0.024$; Supplementary Fig. 1, Supplementary Table 4).

Discussion

In this study, we show that first instar larvae of the mantidfly spider egg predator, *Dicromantispa sayi*, can detect and respond to substrate-borne spider cues in the absence of spiders and may make specific distinctions between different spider life stages. In Experiment 1, when given a choice between filter paper with spider silk and excreta (from adult female, adult male, or juvenile spiders) versus a no cue control, larvae spent more time on the side of the arena with spider cues, and this effect was strongest with male spiders. In Experiment 2, when larvae were given direct choices between cues from different types of spiders (all possible combinations of cues from adult females, adult males, and juveniles), we found that larvae spent more time on filter paper and exhibited more phoretic behavior with cues from adult females and adult males compared with cues from juveniles. However, when given a choice between adult male and female cues, the larvae showed no heightened attraction to one sex
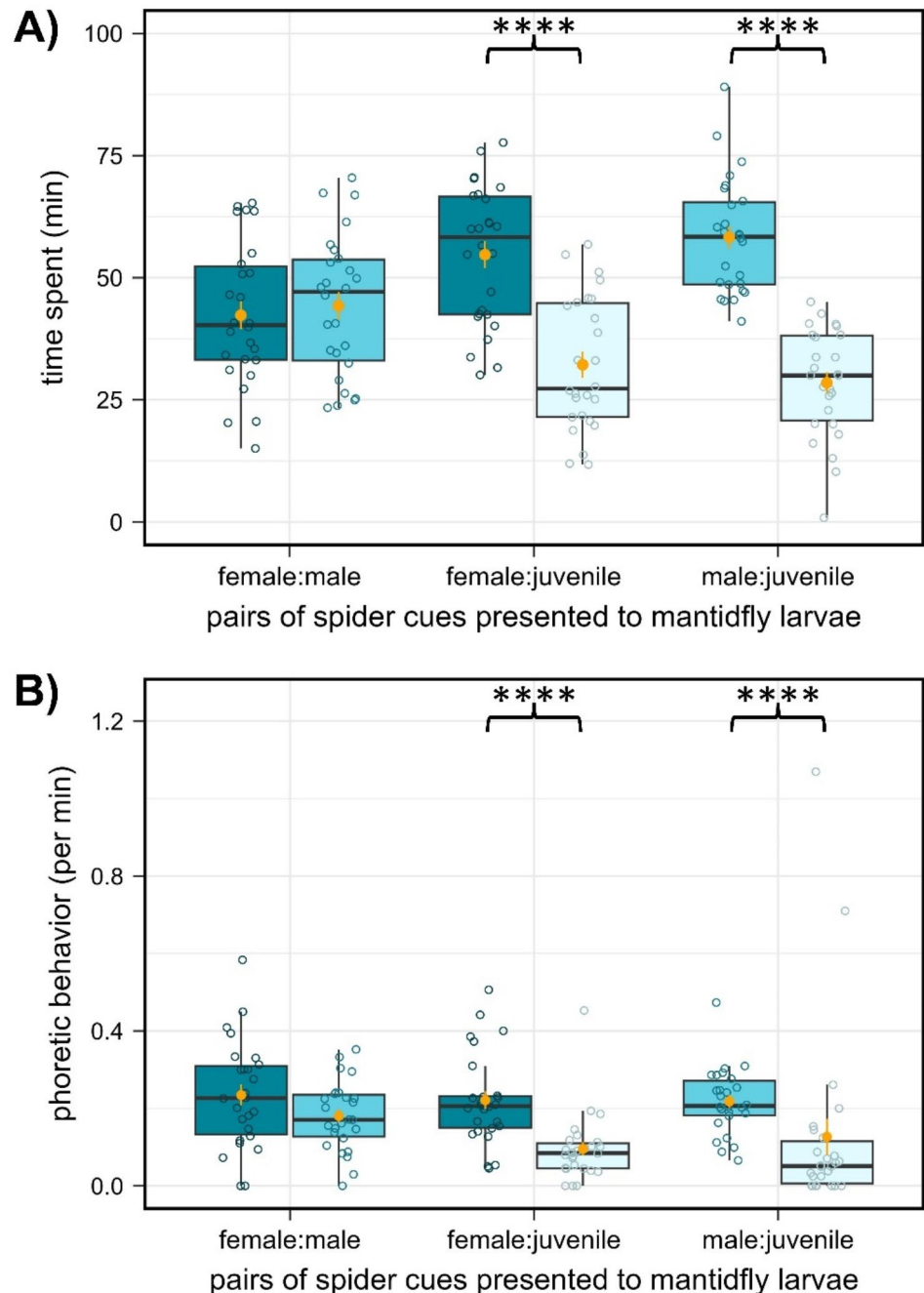
Fig. 4. Results of Experiment 2 where mantidfly larvae (*Dicromantispa sayi*) were given the choice between filter paper halves with different pairs of spider cues (from adult female, adult male, and juvenile *Habronattus trimaculatus* spiders) indicating **A**) time first instar mantidflies spent on each side of the testing arena in minutes, and **B**) the rate of phoretic behavior per minute on either side of the arena. Light orange points with error bars indicate mean and SE, respectively, while boxplots show the median, upper and lower quartiles, and the maximum and minimum values. Open circles represent raw data. "****" indicates p < 0.0001.

over the other. Here, we discuss how such early life decisions in this tiny, understudied spider egg predator may be tied to maximizing their lifetime fitness.

While we can confidently conclude that mantidfly larvae responded to spider cues in our experiments, more work needs to be done to determine whether they were responding to silk draglines, spider excreta, or something else left behind on the filter paper by the spiders. During our experiments, we observed larvae investigating and following dragline silk laid by spiders on the filter paper, suggesting that either tactile or chemical cues present on

	Time spent mean ∓ se (min)	95% CI	F	df	*p*
Female vs. male (n = 26)			0.23	1,50	0.635
Female cues	42.3∓3.08	[35.5–49.2]			
Male cues	44.2∓3.08	[37.4–51.1]			
Female vs. juvenile (n = 26)			34.13	1,50	**< 0.0001**
Female cues	54.7∓2.88	[48.4–61.1]			
Juvenile cues	32.2∓2.88	[25.8–38.6]			
Male vs. juvenile (n = 26)			88.13	1,50	**< 0.0001**
Male cues	58.3∓2.42	[52.9–63.7]			
Juvenile cues	28.5∓2.42	[23.1–33.9]			

Table 2. Results of Experiment 2 where mantidfly larvae (*Dicromantispa sayi*) were given the choice between two halves of filter paper, each side treated with cues from a different type of spider (adult female, adult male, or juvenile *Habronattus trimaculatus*). Results shown are the amounts of time the mantidfly larvae spent on each half of the filter paper in minutes.

	Phoretic behavior (freq) mean ∓ se	95% CI	χ2	df	*p*
Female vs. male (n = 26)			2.063	1	0.151
Female cues	8.92∓1.14	[6.68–11.2]			
Male cues	6.99∓0.93	[5.18–8.80]			
Female vs. juvenile (n = 26)			47.27	1	**< 0.0001**
Female cues	10.66∓1.56	[7.60–13.7]			
Juvenile cues	2.64∓0.48	[1.70–3.57]			
Male vs. juvenile (n = 26)			56.07	1	**< 0.0001**
Male cues	13.12∓2.85	[7.53–18.7]			
Juvenile cues	2.32∓0.59	[1.16–3.47]			

Table 3. Results of Experiment 2 where mantidfly larvae (*Dicromantispa sayi*) were given the choice between two halves of filter paper, each side treated with cues from a different type of spider (adult female, adult male, or juvenile *Habronattus trimaculatus*). Results shown are the number of times each mantidfly larvae exhibited phoretic behavior.

silk may be what drove the patterns in our results. In nature, such silk draglines may be a reliable indicator that a potential spider host is near. Male spiders across taxa are known to use female dragline silk, and its associated chemical cues, to locate and determine the age, diet, and mating status of potential female mates[27,35,36], so it may not be surprising that mantidfly larvae can also detect these same cues from female spiders. However, in our study, mantidfly larvae also responded strongly to substrate-borne cues left behind by both male and juvenile spiders. Much less is known about the nature of any cues produced by juvenile and male spiders[37,38], but our work suggests that there are indeed informative cues available for any receivers that might be paying attention.

We also do not yet know whether the mantidfly larvae in our study were responding to tactile cues or using gustation or olfaction when navigating the test arenas. Our exploratory analyses suggest that their initial choices of which side of the arena to explore were not driven by olfactory cues that they may have picked up from the arena's neutral zone alone (without touching the substrate), as they did not show any biases towards one side of the arena or the other at the start of the trial. Any biases that they expressed for one side of the arena over the other were only apparent after they had time to make contact with and explore the filter paper. More work is clearly needed to tease apart the sensory mechanisms that these larvae are using to respond to substrate-borne spider cues.

Our results showing an apparent preference by mantidfly larvae for cues from adult spiders compared with those from juveniles may suggest that these larvae are making careful distinctions between spider types when seeking out hosts. This is consistent with a large body of work showing juveniles of other animals have particular host preferences[4,39,40]. However, there is also the alternative possibility that the mantidfly larvae were simply responding to the amount of silk (or other cues) present on the filter paper in our experiments. Adult spiders in our experiments were more active than juveniles, and therefore may have elicited a stronger response from the mantidfly larvae simply because they laid down more silk draglines as they were actively moving around the filter paper. The three types of spiders tested (females, males, juveniles) also differ in mean body size (mean mass ± SE (mg): females (19.97 ± 1.02), males (11.90 ± 0.34), juveniles (4.74 ± 0.22)) and it could be that larger spiders lay down more cues than smaller individuals; here again, mantidfly responses could be influenced by the quantity of cues left behind. Now that we know that mantidfly larvae respond to cues from all of these groups of spiders, we can use further manipulative experiments to assess whether it is the quality or quantity of these cues (or both) that matter to mantidfly larvae.

	Costs/risks	Benefits
Adult females	An adult female may have already laid all of the eggs that she will in her lifetime	An adult female may offer the most direct path to a fresh egg sac, particularly if she is gravid Even if she is not gravid, she may return to a previously laid egg sac that she is guarding Even if she has not yet mated, she will likely attract male suitors and will likely lay eggs soon after mating
Adult males	An adult male may not be successful in delivering a mantidfly larvae to a female if his mate search or courtship efforts are unsuccessful Even if he leads her to a female spider, the mantidfly larvae must successfully move from the male to the female spider, or from the male spider to the nest of the female	Because adult male spiders spend much of their time searching for females, a mantidfly larvae that boards a male spider might be delivered directly to a female that will mate and lay eggs Even if the male is cannibalized during courtship, a mantidfly larvae has the potential to transfer to the female
Juveniles	A mantidfly larva may have to remain and survive on the juvenile spider for a long time (until the spider matures and is able to deliver the larva to eggs) The mantidfly larva will have to navigate risks associated with remaining on the spider through molting events	Small juvenile spiders are often found in high abundance; this may make them the most accessible way for mantidfly larvae to disperse from their siblings Juvenile spiders may face high risk of predation from larger spiders, but mantidfly larvae have the potential to transfer to these larger hosts during predation events; however, any such benefit may be minimal or negligible if the spider host's main predators are non-spiders Mantidfly larvae that board juveniles may be able to overwinter on these spiders (during times of the season when adult male and female spiders die)

Table 4. Potential costs/risks and benefits to mantidfly larvae choosing to board different spider sexes and life stages.

Regardless of whether mantidfly larvae in our experiments responded to the quality or quantity of spider cues present, our results suggest that larvae will still most likely end up responding more strongly to cues from adult spiders (compared with juveniles). The lack of any distinction between adult male and female spiders is consistent with mantidfly boarding rates reported in the literature. In a lab setting, *D. sayi* have been shown to successfully board females and males in relatively equal numbers in no-choice tests[18]. In the field, surveys on two mantidfly species (*Leptomantispa pulchella* and *Dicromantispa sayi*) found larvae on both sexes in relatively equal numbers[18,22]. However, in contrast with our results, juvenile spiders were found with *L. pulchella* larvae more often than adults[22], and *D. sayi* larvae are found equally on adults and immatures[18]. Even if mantidfly larvae are most attracted to cues from adult spiders, they may not be very selective in nature or may frequently board juvenile spiders when those juveniles are found in higher abundance.

As we move towards experiments to further understand the specific cues that mantidfly larvae use to make decisions, it is important to consider the possible costs and benefits that may be associated with different choices (summarized in Table 4). Adult female spiders offer the most direct pathway to egg sacs if they are gravid, unmated, or currently tending to egg sacs, but also are a dead end if they have already laid all possible eggs in their lifetime. Boarding an adult male could help lead mantidfly larvae to females directly, but host transfer may be difficult and has only been observed when the male is cannibalized by the female[18]. Males successfully coming into contact with females is also not guaranteed[41,42]. Juvenile spiders are arguably the most risky potential hosts due to delayed time to maturation or egg sac encounter, the need to navigate juvenile spider molting[18], and other additional mortality risks to juvenile spiders (i.e., mismolting[43–45], extreme temperature[46]). In ideal lab conditions, larvae that successfully boarded immature female *Rabidosa rabida* spiders only had a 12.5% success rate of adult emergence[18]. However, a possible benefit to boarding juvenile spiders might be, counterintuitively, the heightened risk of predation of the juvenile host by other spiders; if the mantidfly larva is not consumed, it may actually have the opportunity to transfer to the larger spider predator[47]. Additionally, if the seasonality of mantidfly emergence occurs at a less than ideal time, mantidflies may also be able to overwinter on boarded spiders and maintenance feed on hemolymph for up to a year, as many spiders overwinter as juveniles or subadults[18,22,25,48]. Alternatively, swift dispersal from the hatching site, and thus less host choosiness, may be the most advantageous strategy for first instar mantidflies that have extreme competition for resources with hundreds or thousands of siblings simultaneously emerging (as seen in nematodes[49]). This alternative explanation could help explain why the mantidflies in our study made no fine distinctions between adult female and male spider cues. While it has been little studied, it seems likely that survival as a mantidfly larva is incredibly difficult regardless of the spider host that an individual finds, if they find a host at all.

Ultimately, strategic decisions made by first instar mantidflies to secure resources likely affect their survival, adult body size, and mating success as adults. We showed here that mantidflies are capable of detecting spider cues in the absence of spiders and may also make distinctions between different spider life stages, although the mechanism they use remains unknown. The costs and benefits of spider host choice decisions outlined here can help us generate hypotheses and design manipulative experiments regarding juvenile decision-making strategies in mantidflies, an understudied group with a unique natural history. As such, they may provide novel insights into the risks and benefits of different early life decisions and how these decisions may shape fitness outcomes.

Data availability

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L.L. and L.T. contributed to conceptualization, design, writing, and revising. L.L. collected and analyzed data. Both authors approve of the final manuscript.

Declarations

Competing interests
The authors declare no competing interests.

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