Potential Predation by the Edaphic Genus *Psilogamasus* (Mesostigmata: Parasitidae), a Natural Inhabitant Predator Mite in the Rhizosphere in Avocado Crops[1]

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Abstract Of the mesofauna of the avocado (*Persea americana*) crop rhizosphere, most predatory mites belong to the order Mesostigmata, although only a few families have been considered for use in biological control programs targeting soil pests. Despite their potential value for agroecological management, this group has been poorly studied. Mites of the Astigmatina cohort (Acari: Sarcoptiformes) are widely used as prey in the mass rearing of various predatory mites that inhabit soil or plants, but their predation capacity has not been thoroughly evaluated. This research aimed to investigate the feeding habits and evaluate the biological control potential of a species from the Parasitidae family, native to an avocado-growing region in Michoacán, Mexico. This article reports on the genus *Psilogamasus* (Parasitidae), a natural soil inhabitant in avocado crops, and investigates its potential as a predator across all developmental stages. Larvae, protonymphs, deutonymphs, males, and females of *Psilogamasus* were exposed to prey mites from the Astigmatina cohort through *in vitro* bioassays. The results showed that immature stages were highly voracious; however, larval and nymphal mortality suggested a need for supplementary food to complete development. Furthermore, females were observed to be the most active in searching for prey, consuming at least 50 more nymphs than males.

Key Words biological control, edaphic mites, natural enemies, natural soil mesofaunae interactions

Avocado (*Persea americana* Miller) is a crop of high economic importance, with Mexico being the world's leading producer, accounting for approximately 45% of global production (Khan et al. 2021, Orozco-Meléndez and Paneque-Gálvez

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2023, Ramírez et al. 2024), and with the state of Michoacán leading national output (de la Vega-Rivera and Merino-Pérez 2021, Ramírez et al. 2024). The expansion of avocado cultivation has been accompanied by increased pesticide use, resulting in environmental concerns such as contamination, biodiversity loss, and the emergence of resistant pest populations (de la Vega-Rivera and Merino-Pérez 2021, Pérez-Solache et al. 2023). In Michoacán, major pests include the beetles *Copturus aguacatae* Kissinger, *Conotrachelus* spp. Schönherr, and *Heilipus lauri* Boheman (Coleoptera: Curculionidae); *Stenoma catenifer* Walsingham (Lepidoptera: Depressariidae) (Luna et al. 2017, Peterson and Orden 2008); the thrips *Neohydatothrips*, *Scirtothrips*, *Frankliniella*, *Arorathrips*, *Caliothrips*, and *Leptothrips*) (Thysanoptera) (Bravo-Pérez et al. 2018); and phytophagous mites such as *Oligonychus persea* Tuttle and *Oligonychus punicae* (Hirst) (Tetranychidae) (Estrada-Venegas et al. 2002, Lara et al. 2017). To develop effective pest management strategies, it is crucial to investigate the ecological interactions of native arthropods within avocado agroecosystems (Subhagan et al. 2020). Mites play a vital role in these systems by regulating populations of other invertebrates (Chaires-Grijalva 2012, Chaires-Grijalva et al. 2016, Walter and Proctor 2013).

The order Mesostigmata includes numerous predatory species that feed on various arthropods and small invertebrates, such as nematodes, collembolans (springtails), insects, and other mites (Berndt et al. 2004, Moreira and de Moraes 2015, Navarro-Campos et al. 2012, Walter and Proctor 2013). These organisms, with great potential for biological control, have been little studied (Knapp et al. 2018, Moreira and de Moraes 2015). Members of the family Phytoseiidae are the most well known within this group for their recurrent use in biological control programs (Knapp et al. 2018). Phytoseiid mites effectively regulate the populations of mites and insects that attack the foliage of cultivated plants (Calvo et al. 2015); however, most free-living mesostigmatid predatory mites are edaphic and abundant soil inhabitants (Walter and Proctor 2013). The families Laelapidae, Blattisocidae, Ascidae, and Parasitidae also have potential as biological control agents due to predatory activity observed in the laboratory and the field against different harmful organisms (Castilho et al. 2015, Messelink and Van Holstein-Saj 2008, Navarro-Campos et al. 2012). Despite being little used in agriculture, some species of genus *Stratiolaelaps* (Laelapidae) are marketed by at least 11 companies in the Americas and Europe for the control of soil pests in greenhouse vegetables (Moreira and de Moraes 2015).

The family Parasitidae consists of 15 genera and 130 species (Yao et al. 2020), commonly found in soils with high organic matter content, compost, leaf litter, and mosses and in bird nests or small mammal shelters (Rueda-Ramírez et al. 2019, Szafranek et al. 2013). They are distributed worldwide and, in their final nymphal stage, are phoretic, using some insects as temporary transport without causing harm (Hofstetter et al. 2009). However, species such as have been observed feeding on the eggs and larvae of the beetles with which they disperse (Chaires-Grijalva et al. 2016, Hofstetter et al. 2009). Although this family has not been economically exploited, promising members have been evaluated for use in sustainable agriculture.

Parasitus bituberosus Karg is considered a potential agent for the biological control of insects such as the flies *Heteropeza pygmaea* Winnertz (Diptera: Cecidomyiidae) and *Lycoriella solani* Winnertz (Diptera: Sciaridae) as well as nematodes and other mites that damage the mycelium of cultivated mushrooms in

Europe (Al-Amidi and Downes 1990, Szafranek et al. 2013). In Colombia, the potential of *P. bituberosus* for the control of thrips and nematodes has been evaluated (Rueda-Ramírez et al. 2019). Another predatory species, *Parasitus fimetorum* (Berlese), has been tested for controlling tomato root-knot nematodes in the laboratory and greenhouses in Egypt (Heikal 2020). However, for most Parasitidae spp., different aspects of the ecological processes that modulate their reproductive habits, feeding behaviors, and interactions at various trophic levels remain unknown, particularly in relation to other organisms present in soils without anthropogenic alterations and those under agricultural management in Mexico. From an entomological perspective, some members of the Parasitidae show great potential as natural enemies of various invertebrates and edaphic arthropods (Chaires-Grijalva et al. 2006).

This research was conducted to understand the feeding habits and evaluate the biological control potential of a species from the family Parasitidae native to an avocado-growing region in Michoacán, Mexico. In this study, we established conditions for the reproduction of *Psilogamasus* sp. (Parasitidae: Mesostigmata) individuals and designed an *in vitro* cointeraction system between the predator and mites from the cohort Astigmatina (Acaridae). The cointeraction system allowed us to evaluate predation potential across three developmental stages. This study also represents the first report of the genus *Psilogamasus* in Mexico.

Materials and Methods

Sampling site. An avocado orchard (*Persea american*a v. ‘Hass’) exhibiting symptoms of physiological damage caused by thrips and phytophagous mites was selected for this study. The orchard is located within the avocado-growing region of Michoacán, Mexico, in the municipality of Salvador Escalante (19°20′13.2′′N, 101°38′55.5′′W) at an elevation of 2,328 m above sea level. In 2023, four soil samples were collected monthly (e.g., November, February, May, and June). The months were strategically selected to coincide with periods of high mesofauna activity because cold and/or humid conditions reduce the number and activity of native populations of thrips and mites in the soil (Table 1).

Mite collection. To collect the organisms under study, agricultural soil samples ($n = 5$ per visit) were taken from the avocado orchard. Soil was collected at a distance of 1.5 m from the trunk and to a depth of 20 cm, including the leaf litter layer. For transportation to the laboratory, the samples were placed in double-sealed polypropylene bags (26.8 cm × 27.3 cm, Ziploc®, S.C. Johnson & Son, Inc., Racine, WI). Samples were processed and examined in the Laboratorio de Entomología, Universidad Michoacana de San Nicolás de Hidalgo (LE-UMSNH). Mites were carefully recovered using Berlese funnels. After an initial morphological evaluation, individuals were separated using a fine soft-bristled brush and a dissecting needle under a stereomicroscope at 40×.

Establishment of breeding stock and identification. The Mesostigmata mites were fed using a previously established breeding protocol at the LE-UMSNH for soil mites (Astigmatina: Acaridae), based on the “box-in-box” methodology developed by Jung et al. (2018), with modifications proposed by Esquivel-Ayala et al. (2024). Predators and astigmatine mites were kept in airtight containers measuring 10 cm in diameter × 5 cm in height, with an opening in the lid covered by polyester mesh (100 μm). Each container was filled with 12 g of plant substrate

Table 1. Meteorological data of Salvador Escalante, Michoacán, Mexico, during the sample collection.

	Parameter			Days of Sampling		
Month	Temp. maximum (°C)*	Temp. minimum (°C)*	Rainfall (mm)**	Date	Temp. maximum (°C)*	Temp. minimum (°C)**
February	28.96	8.43	0	21	29	11
March	31.26	10.35	11.02	22	33	10
May	29.29	12.84	31.82	9	32	10
June	33.30	15.33	60.81	7	31	15

* Temperature data source: https://www.accuweather.com/es/mx/salvador-escalante/.

** Rainfall data source: https://smn.conagua.gob.mx/es/.

containing 30% organic matter, 7 g of vermiculite, and 1 g of wheat bran were moistened with water by using a spray bottle. Colonies were maintained at room temperature in the dark and monitored every 24 h. The growth of the populations was followed. Some specimens, as well as the mites that were perishing, were preserved in 70% alcohol for mounting on slides with Hoyer's liquid. Subsequently, the mites were identified to order and family levels by using mainly the morphology keys established by Lindquist et al. (2009), Kazemi et al. (2013), Hrúzová and Fendõa (2018), and Yao et al. (2020).

Predation bioassays. To determine the preference and daily prey consumption of the predatory mites, 20 nymphs were first offered over a period of 5 d to 5 adult mites, with monitoring every 8 h to observe consumption. It was determined that within 8 h, five nymphs offered as prey had been consumed. At approximately 16 h, between 6 and 11 nymphs had been preyed upon. At 24 h, between 6 and 12 nymphs had been consumed. Consequently, it was decided to offer 15 nymphs as prey for the subsequent bioassays. The adults of the astigmatine mites were not preyed upon; thus, this stage was excluded from the experiments. The predation preference and potential of the Mesostigmata mites were evaluated in an *in vitro* interaction system at an ambient temperature of 24°C, as described below.

In a Petri dish measuring 1 cm in height and 1.5 cm in diameter, a homogeneous mixture (M) of plaster of Paris, charcoal, and water was evenly spread across the base of the dish to a height of approximately 3 mm. Once the mixture hardened, 2 g of vermiculite combined with a plant–soil substrate (1:1, v/v) was added (Fig. 1). Seven predator pairs (female and male) were confined and monitored every 8 h until oviposition occurred. Eggs were then individually transferred to a new predation arena (*in vitro* system). The development of 20 eggs of the same age, laid within a maximum interval of 16 h, was monitored.

When the predator larvae hatched, 10 nymphs were provided as prey. Upon transitioning to the protonymph and the deutonymph stage, respectively, 15 astigmatine nymphs were offered as prey. Developmental stages were checked every 8 h until the predators reached the adult stage. Daily prey consumption was

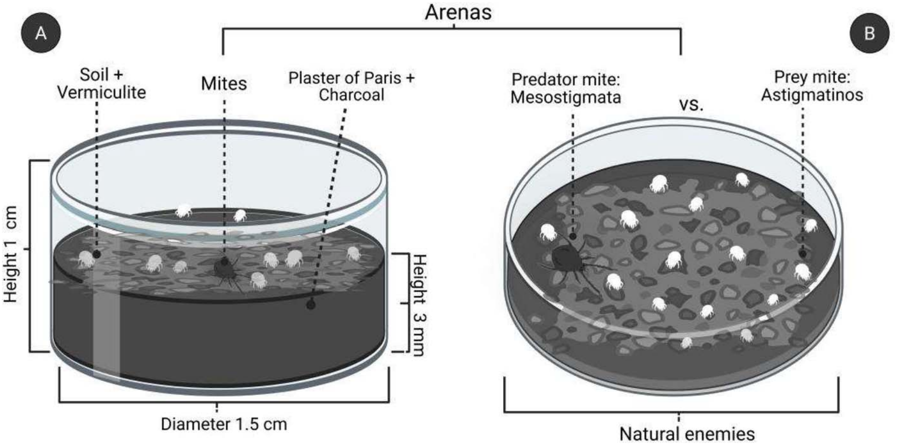

Fig. 1. *In vitro* interaction system between natural enemies of the mesofauna and predation patterns of *Psilogamasus*. (A) Predation scenario in Petri-type dishes. (B) Predation arenas with prey nymphs and predatory mites.

recorded every 20 h. For evaluating prey consumption by adult predators, 10 newly molted, unmated specimens of each sex from an established colony were confined individually. Each predator was provided with 15 nymphs every 24 h. However, because no significant differences were observed in daily evaluations, data were accumulated every 72 h to cover 10 age intervals (30 d). If an astigmatine deutonymph completed its development or entered the hypopial stage, it was replaced with a new nymph.

Statistical analysis. Data were statistically analyzed using SAS® software (SAS/STAT version 9.3, SAS Institute, Cary, NC). With generalized linear models, the variance of quantitative measurements of consumption by developmental stage (larva, protonymph, and adult of both sexes) of mesostigmata as well as the accumulated total of immatures was analyzed. The means were separated with the LSMEANS test ($P \leq 0.01$). Total adult consumption by sex was compared with Student's t test ($P \leq 0.05$). All predation data were expressed as mean ± SE.

Results and Discussion

During the sampling phase, 18 adults and 14 immatures of the predator in total were collected from the avocado orchard. The predator mite was identified as *Psilogamasus* by using the morphological keys of Yao et al. (2020). Currently, there are no records of this genus in Mexico. However, it is speculated that it can be found in North America (Yao et al. 2020). Predation evaluations for females and males showed significant differences in sex ($F = 11.53$; df = 1, 139; $P = 0.0009$), age ($F = 10.54$; df = 9, 139; $P < 0.0001$), and the interaction (sex–age) ($F = 2.37$; df = 9, 139; $P = 0.0157$) of the mite *Psilogamasus* sp. It was found that females consumed more prey than males (Fig. 2). Predation ranges for astigmatine nymphs as assessed were from 15.8 ± 1.5 to 31 ± 2.4 for females, whereas

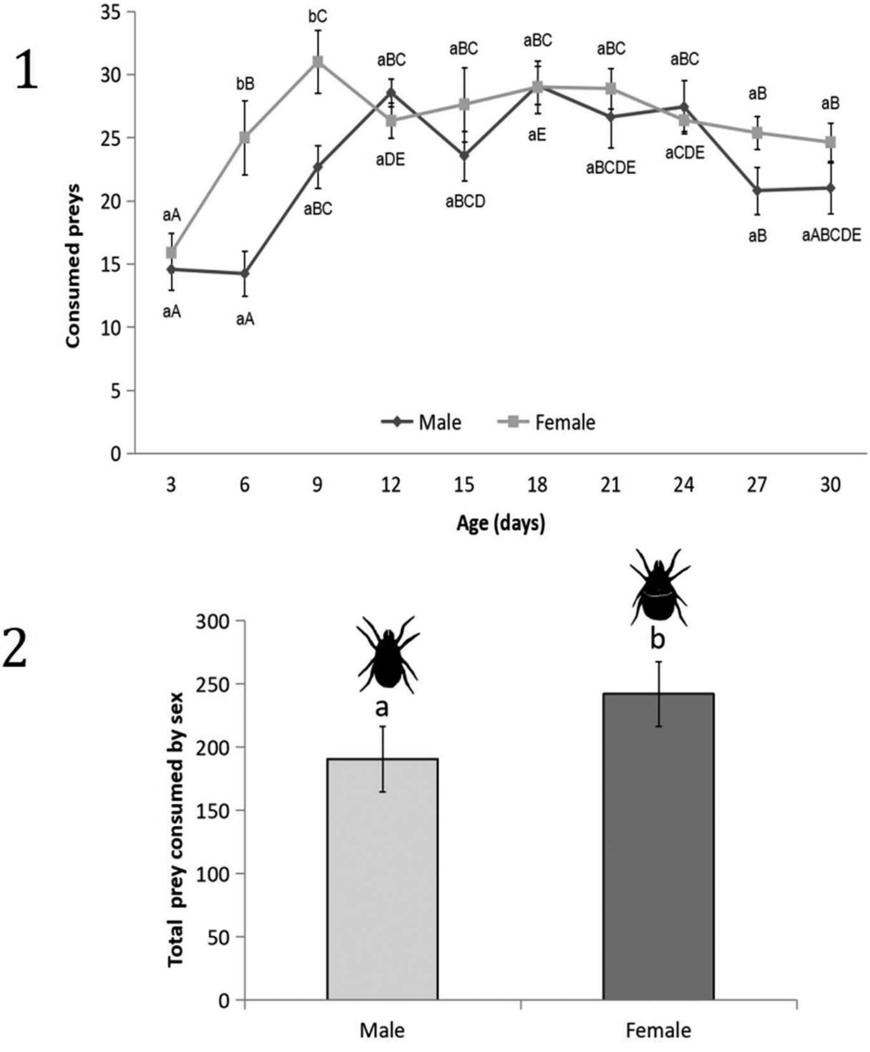

Fig. 2. (1) Number (mean ± SE) of nymphs preyed by adults of *Psilogamasus* sp. every 72 h. Above the error bars, capital letters indicate the comparison between sexes; lowercase letters show the comparison between the ages of male and female. (2) Mean total consumption (±SE) of females and males of *Psilogamasus* sp. 30 d after the experiment.

males preyed on from 14.2 ± 1.7 to 29.1 ± 1.5 prey every 72 h. During the evaluations, it was observed that females lived longer than males. At 9 d of adulthood, females consumed 31 ± 2.4 prey, whereas males consumed 22.6 ± 1.6 (Fig. 2). This result is influenced by the reproductive age of the predator; however, because they were virgin adults, no offspring were produced, demonstrating that *Psilogamasus* sp. does not possess parthenogenetic capacity, as previously reported for other mites of the suborder Uropodina (Mesostigmata) (Bloszyk et al. 2004).

Males consumed significantly more prey than the remainder of the ages evaluated (in 10–20 d). The 3-d-old females consumed significantly less prey (15.8 ± 1.5) than the remainder of the ages evaluated. By contrast, 9-d-old females consumed significantly more prey than 3-, 6-, 27-, and 30-d-old females (31 ± 2.4 versus 15.8 ± 1.5, 25 ± 2.9, 25, 3 ± 1.2, and 24.6 ± 1.4) (Fig. 2), respectively. It is noticeable that predation of *Psilogamasus* sp. on astigmatine mites is greater than *P. bituberosus* feeding on thrips pupae and prepupae, where its consumption was 4.4 in 24 h. (Rueda-Ramírez et al. 2019).

The total accumulated consumption between sexes is significantly different ($F = -2.07$, $P < 0.0552$) (Fig. 2). The cumulative mean of consumption by female *Psilogamasus* sp. was 242.11 ± 18.30 Astigmatina nymphs, whereas the males preyed on 190.67 ± 16.84 in the 30-d duration of the experiment. For males, regarding predation rates, the lowest and highest consumption of prey was 99 and 267, respectively. The consumption of the females ranged between 123 and 311 as a minimum and maximum, respectively. It is remarkable that other published works assessing predation generally do not separate adults by sex given the difficulty of distinguishing females and males and the enormous morphological diversity of mesostigmatines (Baker and Schwarz 1997). Parasitidae spp. studied herein show differences due to the separate podonotal shield of females and the holoventral shield of males (Kaczmarek et al. 2021), allowing separation under a stereoscope.

During development of *Psilogamasus*, significant differences occur with predation by stages ($F = 173.32$; df $= 2, 38$; $P < 0.0001$), age ($F = 1.62$; df $= 5.14$; $P < 0.158$), and in the interaction stage–age ($F = 1.36$; df $= 7, 154$; $P < 0.227$) (Fig. 3). On day 1 of the larval stage, *Psilogamasus* sp. preys on 3.4 ± 0.19 astigmatine nymphs, a significantly lower number than on the remaining days of the same stage and other stages. For day 2 and day 3, consumption increases to 4.05 ± 0.28 and 4.66 ± 0.42, respectively. On the last day of this stage, day 4, predation increased to 5.3 ± 0.59, a significantly higher value than for the remainder of the larval days (Fig. 3).

In the first stage, the highest mortality was recorded, with six larvae dying, two during the third day and four on the fourth day. It has been documented that some families of Mesostigmata are not obligate predators or that they require different food sources during their development (Rueda-Ramírez et al. 2019). Protonymphs of *Psilogamasus* sp. prey on 6.53 ± 0.58 nymphs of Astigmata. This consumption increased significantly on day 2 and day 3, at 7.93 ± 0.54 and 7.84 ± 0.42, respectively. For days 4 and 5, a decrease to 7.28 ± 0.6 and 7 ± 0 occurred. However, there was no statistical difference (Fig. 3). This decrease may be influenced by the proximity to the molt. The mean consumption of the five ages recorded for the protonymphs differs significantly from the remainder of the stages.

There were two deaths during the third and fourth days of the protonymph stage, corroborating the predator's need for other prey or complimentary food sources. The larva and protonymph stages are those with the highest nutritional requirement. During the development of deutonymphs, consumption stands out significantly compared with that of the youngest stages. In the deutonymph stage, *Psilogamasus* sp. preys on 10.81 ± 0.58 nymphs n day 1, followed by 10.09 ± 0.54 on day 2 and 10.54 ± 0.6

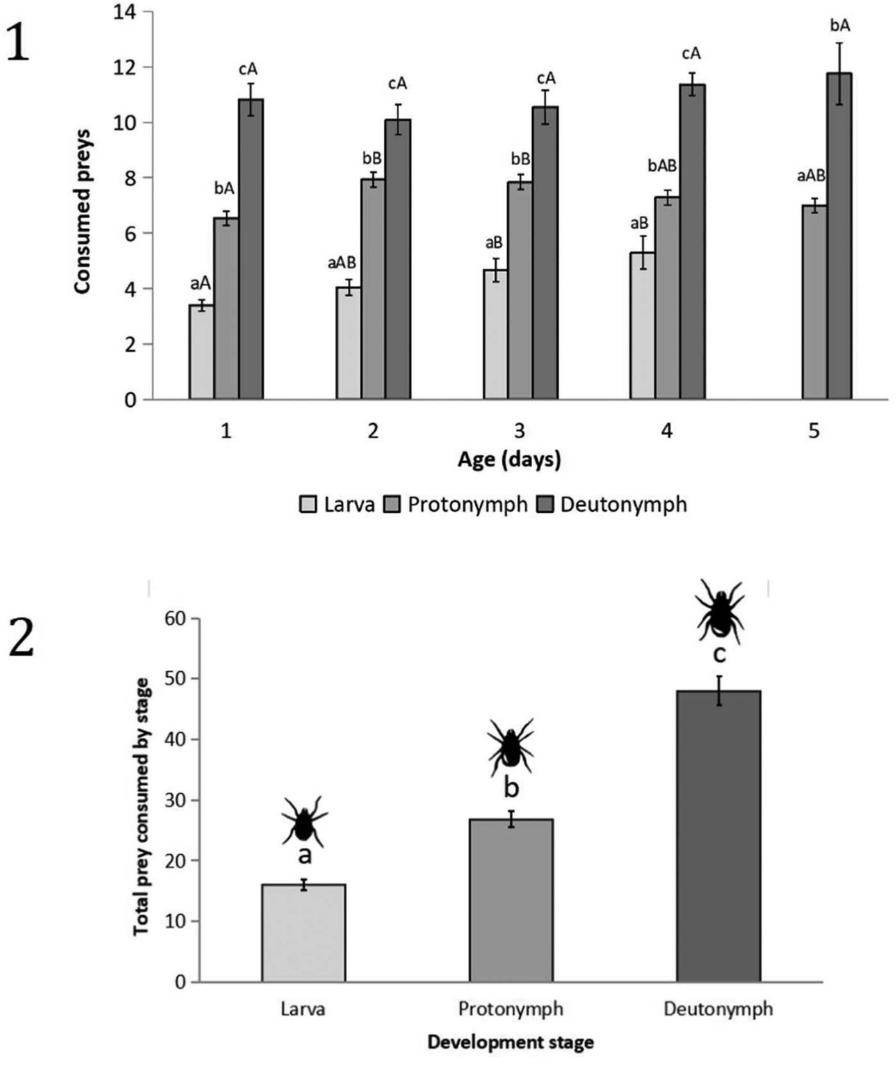

Fig. 3. (1) Daily prey (mean ± SE) per stage of *Psilogamasus* sp. Letters above the error bar indicate the difference between days and lowercase letters between stadia. (2) Total consumption (mean ± SE) per stage of the mite *Psilogamasus* sp. Letters show the difference by state.

on day 3. There is an increase in a prey on day 4 and day 5, at 11.36 ± 0.41 and 11.75 ± 1.1, respectively. However, this predation generates a relevant difference with the remainder of the days in this stage (Fig. 3). The accumulated consumption for the three stages of development differs significantly ($F = 117.03$; df = 2, 38; $P < 0.0001$) (Fig. 3). The larvae of *Psilogamasus* sp. consumed 16.06 ± 0.86 astigmatine nymphs, 26.78 ± 1.3 protonymphs, and 48 ± 2.3 deutonymphs.

This is the first work on the feeding of Parasitidae with astigmatine mites. However, Astigmata is a cohort frequently used for the reproduction of predatory mites (Ballal et al. 2021, Barbosa and de Moraes 2016). Pirayeshfar et al. (2021) mention that astigmatines can support populations of phytoseiids such as *Amblyseius swirskii* Athias-Henriot (1969) when there are low densities of their prey.

Edaphic families such as Rhodacaidae and Laelpidade have successfully reproduced in the laboratory with *Tyrophagus putrescentiae* (Schrank) (Astigmatina: Acaridae) as food, having positive results (with survival $>$78%; Barbosa and de Moraes 2016). In Mexico, Chaires et al. (2006) record of predation by these same families on phytophagous mites, nematodes, collembola, and worms in garlic (*Allium sativum*) crops in Guanajuato.

Soil Mesostigmata are an underrepresented group as biological control agents, highlighting the importance of understanding their potential food resources. Most edaphic species are considered generalists, suggesting that they can be used against various pests or survive periods of scarcity by consuming alternative prey or food sources (Beretta et al. 2004). Herein, astigmatines prove to be a suitable food source for adults of *Psilogamasus* sp.; however, the effect on their biological and demographic parameters of the predator remain unknown, necessitating further evaluation of additional food sources.

This study marks a significant step forward in recognizing the importance of native predatory mites, particularly members of the family Parasitidae, in sustainable agricultural systems. By documenting feeding behaviors, consumption rates across developmental stages, and predator–prey dynamics, this research underscores the versatility and adaptability of *Psilogamasus* sp., demonstrating its ability to regulate populations of soil-dwelling pests effectively. These findings also recognize the importance of native predatory mites, particularly members of the family Parasitidae, in sustainable agricultural systems.

In conclusion, *Psilogamasus* sp. is a highly promising biological control agent, with substantial potential to address persistent challenges in avocado cultivation. Its inclusion in integrated pest management programs has the capacity to significantly enhance agricultural sustainability and reduce dependence on chemical pesticides, fostering healthier agroecosystems in Michoacán. Mexico, as well as other agricultural areas.

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