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First Occurrence of *Paragordius varius* in Different Species of Acrididae in Irapuato, Guanajuato, Mexico

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Abstract

Acridid populations have increased significantly in recent years, causing damage to crops. Having biocontrol tools is important as it will help reduce the use of insecticides. This study reports the presence of *Paragordius varius* in grasshoppers for the first time in Irapuato, Guanajuato, Mexico. Samples were taken from the grass around a water pond. A total of 1,225 grasshoppers were dissected to observe the presence of the worm. Seven species of grasshoppers were identified, with the most abundant species being *Melanoplus differentialis*. The parasitoid *Paragordius varius*, a nematomorph, parasitized male *M. differentialis* in greater numbers. This work is the first record of this parasitoid in Mexico and contributes to the knowledge of the biological control of acridids in Mexico.

Keywords

Grasshoppers, *Paragordius varius*, Parasitism, Nematomorph, Biological Control

1. Introduction

Damage by grasshoppers in the production of staple grains ranges from 50% to 60% [1]. Biological control is an alternative to population management, but few studies have been conducted in the state of Guanajuato in this field. Salas-Araiza *et al.* [2] indicated that *Taeniopoda eques* Burmeister, 1838 (Orthoptera: Acrididae) is parasitized by nematodes, although it is now known to be a Nematomorpha horsehair worm, or Gordian worm, which can reach lengths of 26 cm and

forms a knot inside the main host; in some cases, it reaches up to 2 m length [3].

Gordian worms (Gordiidae: Nematomorpha) are generally parasites of terrestrial arthropods. Once the adult worm reaches this stage, they induce the host to jump into the water, where they emerge from the host, mate, and lay eggs in the water. Upon hatching, the larvae infect a paratenic host, forming a cyst and completing their development when this host is eaten by the definitive host [4].

Paragordius varius (Leidy, 1851) is the most common and widely distributed species in the New World, from North America to the southern continent [5] and Mexico is within its distribution area; adults can measure up to 250 mm [6] and females can lay up to 6.5 million eggs [7]. They are Nearctic in distribution, as noted by Schmidt-Rhaesa *et al*. [8] but there are also reports in the Antilles [5]. Little is known about the nematomorph-arthropod association; the host's ability to move, even when infected, contributes to the dispersal of parasitic nematodes to areas far from water sources, as noted by Looney *et al*. [9].

The objective of this study is to report *Paragordius varius* for the first time in different species of Acrididae in Irapuato, Guanajuato, Mexico.

2. Materials and Methods

The study was carried out at the experimental field of the Department of Agronomy of the University of Guanajuato in El Copal, Irapuato, Guanajuato, Mexico (20˚44'24"N; 101˚20'12"W; 1750 masl; 700 mm annual rainfall; average temperature of 18˚C).

Samples of acridids were taken from different types of vegetation around a water pond using a sweeping net from October 10th, 2023, to November 30th, 2023, on eleven different dates. The sampled vegetation was predominantly Poaceae. The captured grasshoppers were deposited in a jar with 75% alcohol immediately until analyzed in the laboratory. A comparative analysis was carried out with images from the literature [4] [10]. The samples were separated by species and by sex.

The specimens were dissected using fine-tipped scissors introduced through the anus, making a dorsal cut along the midline to the thorax-head junction to expose the hemocoel. The presence of parasites was noted by the naked eye, identifying parasitized individuals by the knot formed by the horsehair worms.

Statistical Analysis

A two-dimensional contingency chi-squared test was performed to evaluate the relationship between male and female and the number of parasitized individuals of the species found in October and November. In case of significant differences, standardized residuals were calculated to identify the categories that generated these differences.

3. Results

A total of 1225 specimens of the following acridid species were examined:

Brachystola mexicana (Girard, 1854), *Sphenarium purpurascens* Charpentier, 1845, *Melanoplus differentialis* (Thomas, 1865), *Schistocerca cohni* Song, 2006, *Syrbula admirabilis* Uhler, 1864, *Boopedom diabolicum* Bruner, 1904 and *Hesperottetix viridis* (Thomas, 1872).

M. differentialis was the most abundant species with 514 individuals, followed by *S. cohni* with 398, and *S. purpurascens* with 158 captured individuals. Their presence was noted since October 10th. On November 4th, *M. differentialis* peaked with 222 specimens collected. This period is when adults mate and oviposit, so a lot of activity was detected in the sampling sites.

Parasitism

Paragordius varius (Nematomorpha: Gordiida) was identified parasitizing the collected acridids; juvenile stages are cream-colored and coiled, forming a knot as noted by its common name. Several juveniles can be present within a single grasshopper, typically located near the Malpighian tubules, with other organs like the digestive system remaining unaffected (Figure 1).

Figure 1. Left: *Taeniopoda eques* (Acrididae) and a *Paragordius* that emerged from the grasshopper. Right: Immature *Paragordius* lodged in the Malpighian tubules of a grasshopper. Irapuato, Gto., Mexico. 2023.

In October, the species recorded with parasites were *B. diabolicum*, *B. mexicana*, *M. differentialis*, and *S. purpurascens*. There were significant differences in the number of parasitized individuals by sex ($X_{(0.05, 3)} = 10$, $p < 0.018$); according to standardized residuals, male *M. differentialis* contributed the most to the observed differences (Table 1).

In November, the parasitized species were *B. diabolicum*, *B. mexicana*, *M. differentialis*, *S. purpurascens*, and *S. cohni*. Significant differences were observed ($X_{(0.05, 4)} = 56.95$, $p < 0.0001$) in the females of *B. diabolicum*, *B. mexicana*, *S. purpurascens*, and *S. cohni*, with *S. purpurascens* being particularly notable.

The development cycle of grasshoppers is around 90 days, so the infection possibly occurs in the 4th nymph stage, allowing *P. varius* to complete its 30-day development [3].

Schmidt-Rhaesa *et al*. [8] indicated that the juvenile stages are white yellowish in color, the adult stage is dark brown, and they can reach 122 mm in males and 220 mm in females; these same authors reported *Gryllus assimilis* (Fabricius,

1775), *G. pennsylvanicus* Burmeister, 1838 and *Nemobius fasciatus* (De Geer, 1773) (Orthoptera: Gryllidae) as hosts, and Ephemeroptera, Heteroptera, and Diptera orders as hosts of juveniles. The infection process of the main host requires that the aquatic host (paratenic host) gets infected by the larvae and forms a cyst, which can survive up to 30 days on vegetation once the paratenic host dies. The main host ingests the vegetation with the cyst, continuing the life cycle of the Gordian worm [11].

Table 1. Parasitism of acridids by *Paragordius varius*. El Copal. Irapuato, Gto. Mexico. 2023. (m = male; f = female).

Date	*B. mexicana*		*S. purpurascens*		*M. differentialis*		*S. cohni*	
	Male	Female	Male	Female	Male	Female	Male	Female
oct/10/2023	0	0	0	0	0	0	0	0
oct/15/2023	0	0	0	0	0	0	0	0
oct/23/2023	0	0	0	0	0	0	0	0
oct/30/2023	0	0	0	0	0	0	0	0
oct/31/2023	1	0	0	1	0	7	0	0
nov/04/2023	0	1	0	2	67	0	0	0
nov/06/2023	0	0	0	0	54	2	3	0
nov/14/2023	1	0	0	0	29	2	4	0
nov/18/2023	0	0	0	0	0	0	0	0
nov/23/2023	0	0	0	0	0	0	0	0
nov/30/2023	0	0	0	0	0	0	0	0
Total	2	1	0	3	150	11	7	0

This research highlights *M. differentialis* due to the larger number of parasitized specimens, with 161 (31.3%), and males showing the highest number, with 93.1% of the total parasitized individuals for that species (**Figure 1**).

4. Discussion

This is the first report of nematomorphs parasitizing grasshoppers, contributing to the knowledge of natural enemies of the Acrididae family in Mexico. Ponton *et al*. [10] noted that *Paragordius tricuspidatus* Dufour, 1828 (Nematomorpha: Gordiida) escapes from the primary host by causing it to jump into the water. The movement of the cricket in the water attracts fish and frogs that ingest it; the worm then emerges from the fish or frog to escape their digestive tract. Thus, the horsehair worm continues its cycle in its aquatic stage where it mates and lays eggs.

Juvenile stages are obligate parasites of terrestrial arthropods. Even when infected, the host's mobility helps disperse parasitic nematodes to distant locations from water sources [9].

The presence of this parasitoid was higher in males of *M. differentialis* and *S.*

cohni, indicating a preference for this sex (Figure 1). It is possible that the feeding habits of the males occur in areas where the infective stages of the worm, such as cysts left by the secondary host, predominate. This is similar to the parasitic nematode *Howardula aoronymphium* Welch, 1959 and its insect host *Drosophila falleni* Wheeler, 1960; both organisms are attracted by the odor of the mushroom *Agaricus bisporus* (J.E. Lange) Imbach, 1946. When they coincide on the mushroom, the larvae of the fly are infected by the nematode. The main attractant for the parasitic nematode is identified as the carbon dioxide emitted by the mature mushroom [12].

Possibly within the host, the larvae penetrate the walls of the digestive tube and insert themselves in their definitive location; in the present study, they were observed in the posterior part of the mesenteron in the region of the Malpighian tubules.

Parasitic nematodes locate the host by smell, just like the insect host [12]. This helps explain the different feeding preferences between male and female *M. differentialis*. This inference might be useful, but given the limited information available about this worm, this hypothesis still needs to be confirmed.

5. Conclusion

This study was conducted in central Mexico, where the problem with grasshopper infestations has significantly worsened, causing damage to crops, fruit trees, and urban greenery, and even invading buildings. This study reports seven species of acridids, with *M. differentialis* being the most abundant, followed by *S. cohni*. Knowledge of beneficial insects associated with acridids will help reduce insecticide applications. Therefore, this first report of *Paragordius varius* (Nematomorpha) parasitizing acridids is a useful tool for continuing the study of biocontrol management strategies for acridid populations in the field. Notably, male *M. differentialis* showed the highest parasitism, possibly because males prefer to feed on certain plants located around the pond. This research contributes to the understanding of natural enemies for acridid control.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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