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Original article

Is *Hormogaster elisae* (Oligochaeta, Hormogastridae) a predator of mites and springtails?

M. Gutiérrez López, J.B. Jesús Lidón, D. Trigo Aza, D.J. Díaz Cosín*

Dpto. Zoología y Antropología Física, Facultad de Biología, Universidad Complutense de Madrid, 28040 Madrid, Spain

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Abstract

The aim of this work was to determine whether the endogeic earthworm *Hormogaster elisae* [1] is involved in the active or passive predation of microarthropods at El Molar (Madrid, Spain). Different techniques were employed to study the gut content, and the casts of *H. elisae* earthworms cultivated in the laboratory. The casts consisted mainly of mineral particles and plant remains as well as a few microarthropods, nematodes and their remains. The gut contents were similar in composition, although no microarthropod remains were found, except for a single springtail (order Poduromorpha) in one earthworm's gizzard. The results suggest that *H. elisae* may accidentally ingest microarthropods along with soil. The microarthropods found in the casts may have colonized them after their deposition since none were found in isolated casts.

Keywords: *Hormogaster elisae*; Acari; Springtails; Predation; Earthworm casts; Gut content

1. Introduction

There is no consensus in the literature about the positive or negative relationships between earthworms and microarthropods. In a previous paper, Gutiérrez et al. [10] reported a negative relationship between the earthworm *Hormogaster elisae* [1] and microarthropod abundance at El Molar (Madrid, Spain). Some authors [17,14] have reported similar negative relationships between earthworms and several microarthropods species. Anyway, there are also some studies that show a favorable impact of earthworms on microarthropods [15,12,21].

Brown [4] reviewed the positive or negative effect of earthworms on microarthropods reported in different papers, concluding that in most cases the main effect is a reduction in microarthropods density and species diversity, unless they are able to use the earthworm processed residues, as casts components. McLean and Parkinson [16] found that earthworms activities had varying effects on abundance of oribatid species and other groups of microarthropods, maximum abundance tended to occur at intermediate levels of earthworm activity. Maraun et al. [13] and Scheu et al. [23] also found varying effects on microarthropods depending on the group.

The mechanisms underlying the negative influence of earthworms on microarthropods observed at El Molar may be multiple, involving physico-chemical perturbations, competition or predation. One hypothesis for explaining this negative relationship suggests that earthworms may be predators of microarthropods because remains of their cuticles have been found in the gut of some earthworm species [19]. The food habits of earthworms are very heterogeneous [18]

* Corresponding author.
E-mail address: dadico@bio.ucm.es (D.J. Díaz Cosín).

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since the soil they ingest contains not just organic matter but also microorganisms [5] and soil fauna. Wolter and Scheu [26] report there is evidence that earthworms ingest bacteria [8], fungi [24], protozoa [3,20], algae [19] and nematodes [5].

McLean and Parkinson [16] indicate that it has not been sufficiently well documented whether the consumption of microarthropods by earthworms is active or passive, and propose that this is probably not the main mechanism determining the relationships between these groups. Although the microarthropods do not appear to be an important part of the earthworm diet, it is not sufficiently known if an active predation exits. In fact, the observed reduction in the size of microarthropod populations in the presence of earthworms could be due to the active or passive ingestion of the former, independent of whether or not they are digested.

Each species of earthworm has its own food substrate preference [6]. Some authors have suggested that earthworms may select different groups of soil organisms as part of their diet nutritional [4]. It is known that *H. elisae* normally prefers to ingest the smallest soil fractions and the species' enzyme profile gives some clues as to its diet, but until now few data have been available. The majority of endogeic earthworms have weak gut enzyme systems and usually establish symbiotic relationships with the soil microflora in order to digest some organic compounds [11]. This has been studied in *H. elisae*, which has a weak glycolytic enzyme system; it therefore probably makes use of the digestive enzymes of the microflora it ingests [25,9]. In addition, one of the main enzymes detected in the gut of *H. elisae* by Garvín et al. [9] is *N*-acetyl-glucosaminase. This can attack substrates such as chitin, which is found in fungal cell walls, roots and arthropod exoskeletons. This might provide an insight into the diet of this species, and raises the question of whether microarthropods can be digested.

This work is part of a wider project that investigates the interactions between *H. elisae* and soil acari and springtails. The aim was to determine whether *H. elisae* prey on microarthropods, one of the possible hypotheses that could explain the negative relationship seen before between these groups in laboratory cultures by Gutiérrez et al. [10]. Gut content and casts were examined in order to determine whether ingestion of microarthropods occurs, and to test if microarthropods colonize casts after their deposition.

2. Materials and methods

The gut contents and fresh casts of specimens of *H. elisae* were inspected for acari and springtails. Soil and earthworms were collected at El Molar (UTM 30TVL5210), by digging and hand sorting. *H. elisae* is an endogeic, oligohumic earthworm endemic to the center of the Iberian Peninsula [9]. The soil of El Molar is rich in sand and poor in organic matter and its physical and chemical characteristics are shown in Table 1.

Six soil samples of 400 g were used to extract the microarthropods before the beginning of experiments, in order to evaluate the microarthropod community composition and to know the density of the potential preys. Microarthropods were extracted by the Berlese–Tullgren method, preserved in Scheerpeltz-s liquid (alcohol 70°, glycerol and acetic acid) and counted and identified under a stereomicroscope.

Twelve experimental microcosms were made. They consisted of hermetical plastic containers (12 cm internal diameter and 6 cm height) with 400 g of soil (which contain the natural community of microarthropods) brought to 20% moisture. One earthworm weighing 2–3 g was placed inside each microcosm. The microcosms were kept for 21 days at 13 °C in a dark chamber. Fresh casts were collected daily from the surface of the microcosms and conserved in 70% alcohol. After this time, six earthworms were then placed in Petri dishes for 24 hours until the gut was fully evacuated. All casts were collected and conserved in the same way. The six remaining earthworms were killed by immersion in boiling water and preserved in 10% formalin prior to dissection. The gut was divided into four sections—gizzard, foregut, midgut and hindgut—and the content of each, removed and preserved in 70% alcohol. *H. elisae* has a well developed tiflosol, especially in the foregut; special care was therefore taken to extract all gut material, cleaning between the folds with a fine paintbrush.

The gut contents and fresh casts were subjected to different techniques to facilitate their analysis. They were first passed through 45–50 μm filters to eliminate

Table 1
Physical and chemical characteristics (mean and standard error) of the soil from El Molar. C: carbon content, N: nitrogen content

Coarse sand (%)	Fine sand (%)	Coarse loam (%)	Fine loam (%)	Clay (%)	pH	C (%)	N (%)	C/N
53.00 ± 7.98	14.15 ± 3.13	3.63 ± 2.48	9.46 ± 3.70	19.67 ± 5.35	6.62 ± 0.49	1.83 ± 0.54	0.16 ± 0.05	11.76 ± 1.16

the smallest soil particles. N-heptane was added to the material retained to separate any possible microarthropod remains. The organic particles were stained with rose bengal medium according to the method of Dash et al. [6]. A stereomicroscope was used to examine all samples for microarthropods (or fragments of them), which were identified to the lowest taxonomic level possible.

3. Results

Table 2 shows the microartropods present in 100 g of dry soil from El Molar before the experiment. This community was enough to represent a potential number of preys for earthworms in the experiments.

A mean of earthworms cast production of 207.12 g during the 21 days of the experiment was calculated, using the rate of cast production (3.18 g cast per earthworm gramme and day) for this species [7].

The composition of the gut content was similar in all sections: a mixture of mineral particles of different size, mucus, plant remains (small roots, small sticks and seeds) and unidentified fragments. The foregut contained larger quantities of material than the hindgut. No trace of any microarthropod remains were seen in any part of the gut, except for a single, whole springtail (order Poduromorpha) in one earthworm's gizzard. The specimen was well pigmented and showed no signs of degradation. Fragments of organic material stained with rose bengal medium were also found, but they were difficult to identify and could not be assigned to any particular group.

In general, the composition of the casts was similar to that of the gut contents, with plant remains and unidentified organic material stained with rose bengal medium. However, microarthropods (both whole bodies and fragments) belonging to different groups were also detected (Table 3). Springtails generally stain well with rose bengal medium, especially the least pigmented individuals. Representatives of the order Poduromorpha and of the family Onychiuridae were the most common; a few individuals belonging to the family Isotomidae were also found. Representatives of the three main suborders of soil acari were also found. Those of the suborder Gamasida mainly belonged to the family Pachilaelapidae or to the genus *Rhodacarus*; there were also some juveniles very difficult to identify. Representatives of the suborder Actinedida were the most abundant and the best stained (especially the juveniles). Members of the families Bdelidae and Tarsonemidae were present. Members of the suborder Oribatida were easily identified even though they stained less well with rose bengal medium. Several different representatives of the cohort Gymnonota (family Oppiidae) and a few genera of the cohort Poronota (such as *Scutovertex*, *Ceratozetes*, *Haplozetes* and *Hemileous*) were identified. Nematodes, which stained very well, were also found in all the casts, as well as unidentifiable material.

The number of microarthropods in the daily-collected casts gradually reduced over the experimental period.

No traces of any microarthropods were seen in any of the casts collected after leaving the worms in the Petri dishes.

Table 2
Mean abundance ($N = 6$) of the number of individuals per 100 g of dry soil taken before the experiment

Isotomidae	22.79
Poduromorpha	16.37
Onichiuridae	0.37
Entomobryidae	0.08
Sminthuridae	0.33
Gamasida	0.87
Acaridida	4.75
Actinedida	3.45
Tarsonemidae	4.08
Oribatida Macropilina	4.66
Oribatida Brachipilina Gymnonota	1.70
Oribatida Brachipilina Poronota	2.5
Other fauna	0.87

4. Discussion

Gutiérrez et al. [10] indicate that, in laboratory cultures, *H. elisae* has a clearly negative influence on the density and structure of El Molar soil microarthropod communities: populations are generally larger when the earthworm is absent. The same has been reported for other species of earthworms [17,14], results varying depending on the soil fauna and experimental conditions. There are also a lot of studies reporting positive relationships between earthworms and microarthropods [15,12,21].

Some papers suggest that earthworms are detritivores, fungivores or microbivores. It has been reported that they commonly ingest soil microflora as well as plant and fungal material along with the soil, although some species seem to have its own food preferences [5].

Some authors [19], however, report the gut contents of a number of earthworm species to contain the remains of microarthropod cuticles as well as fungi, protozoa, nematodes and enchytraeids. It should be remembered, however, that the examination of gut

Table 3
Total number of microarthropods (and mean per microcosm) in (a) isolated casts in Petri dishes, (b) casts collected from the surface of the microcosms, (c) earthworm gut material

Taxonomic groups	Petri dishes casts (a)	Microcosm casts (b)	Gut contents (c)
Total Springtails	0	17 (3.33)	1
Isotomidae	0	1 (0.16)	0
Poduromorpha	0	8 (1.16)	1
Onychiuridae	0	9 (1.5)	0
Total Acari	0	46 (8.16)	0
Gamasida (Paquilaelapidae, *Rhodacarus*)	0	5 (0.83)	0
Actinedida (Bdelidae, Tarsonemidae)	0	20 (3.5)	0
Oribatida (Oppiioidea, *Scutovertex, Ceratozetes, Haplozetes, Hemileous*)	0	21 (3.83)	0
Nematoda	0	66 (11.83)	0
Total fauna	0	118	1

material and casts is only an indirect way of learning about earthworm dietary habits [2]. Migge [17] report that earthworms may function as predators in soil and are likely to ingest egg clutches or even juvenile collembolans or mites, presumable, the strong decline in densities of microarthropods may be partly due to predation of earthworms on early life stages of microarthropods.

The analysis of the gut contents in the present work indicates that microarthropods are probably ingested only sporadically and by accident. They do not seem to have passed through the earthworm gut since none were found in the casts collected in the Petri dishes.

The ingestion of more sclerotized microarthropods, such as acari Oribatida, seems unlikely since the remains of their cuticle would have been found; given their thickness they would be difficult to break down. With respect to softer-bodied, less pigmented microarthropods such as juvenile acari of the suborder Actinedida and the majority of the springtails, it is theoretically possible that earthworms might digest them very rapidly, leaving few traces of their consumption in the gut material. This seems unlikely, however, since some remains would almost certainly have been found in the gizzard and foregut where digestion is still incomplete. To confirm this hypothesis, molecular techniques might be used to identify any chitin or nucleic acids present.

The microarthropods found in the casts probably colonized them after their deposition because the high organic matter content of casts renders them appropriate microhabitats. Salmon and Ponge [21,22], report the attraction of some springtails by earthworms because of several processes: microorganisms are more abundant in fresh casts than in surrounding soil and this can attract the springtails, generally earthworm casts are richer in mineral nutrients (Ca^{2+}, K^{+}, Mg^{2+} PO_4^{3-}, NO_3^{-}) and have a higher content in organic matter than the surrounding soil, earthworms excrete organic compounds such as proteins and glycoproteins, urea, amino-acids, vitamins and glycosides [21]. Some studies showed that microbial biomass and nutrient content of casts first increase but then strongly decline with age, while the first phase might be beneficial to decomposer microarthropods, the changes of food availability and food quality in aging casts might be detrimental since microarthropods have to constantly adapt to changing conditions [17]. This aspect could not be test in this experiment because the casts were collected daily and were all of the same age, but it can be one of the causes of the detrimental of microarthropods in other studies.

The absence of microarthropods in earthworm gut and Petri dishes casts, and their presence in soil casts, suggest it is unlikely that *H. elisae* actively preys on acari and springtails at El Molar, although they may be consumed sporadically—and accidentally—during the earthworm's normal feeding on soil. Cast colonization occurs after their deposition.

The predation of microarthropods by earthworms may be therefore excluded, and for this reason a study of the variation of microarthropods abundance or of the community structure during experiments was unnecessary. The hypothesis of predation does not explain the negative relationships between microarthropods and *H. elisae* previously observed. Other hypothesis, such as competition or physical perturbation, will be considered in future works.

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