



Original Article

Comparative assessment of occurrence of *Monocystis sp.* in wild and cultured varieties of *Lumbricus terrestris* (Linnaeus, 1758)

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Manuscript ID:
RIGJAAR-2025-020605

ISSN: 2998-4459
Volume 2
Issue 6
Pp. 26-31
June 2025

Submitted: 06 May 2025
Revised: 10 May 2025
Accepted: 15 June 2025
Published: 30 June 2025

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Quick Response Code:



Web: <https://rlgjaar.com>



DOI:
10.5281/zenodo.17045743

DOI Link:
<https://doi.org/10.5281/zenodo.17045743>



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Abstract

India, known for its strong agrarian economy, faces significant environmental issues, for which earthworms might offer a feasible solution. The protist *Monocystis*, a unicellular parasite that forms spores, resides in the seminal vesicles of all earthworm species and is associated with parasitic infections that can result in castration. Therefore, the presence of *Monocystis* raises major concerns. This group of gregarine parasites, a member of *Apicomplexa* phylum, lives in the seminal vesicles of earthworms and feeds on their sperm. The aim of this research is to assess the prevalence of *Monocystis* infection across various types of earthworms, with a particular focus on comparing wild and cultured specimens. Earthworms were collected from both natural habitats and cultured environments from reputable sources, and their seminal vesicles were subsequently excised. These samples were then teased in normal saline and examined under a microscope. This study suggests that wild-type earthworms show a considerably greater vulnerability to *Monocystis* infections than their cultured relatives, supporting earlier claims that "all earthworm species are infected by *Monocystis*." The current study provides the initial definitive microscopic documentation of the presence of *Monocystis sp.* in wild populations of *Lumbricus terrestris*, potentially identifying it as *Monocystis lumbrici*, but further analysis is needed to confirm this.

Keywords: Earthworm, *Lumbricus*, *Monocystis*, Seminal vesicles, Smear, Trophozoite

Introduction

Earthworms, belonging to the phylum Annelida and class Oligochaeta, are segmented, burrowing organisms that inhabit terrestrial environments. These creatures are hermaphroditic and primarily consume organic matter found within the soil. They play a crucial role in soil ecosystems and represent a significant portion of the invertebrate population in these habitats. Globally, there are approximately 7,000 recognized species of earthworms, which exhibit considerable variation in morphology (Phillips et al., 2019). These organisms are distributed across all continents, except Antarctica; however, their distribution is inconsistent worldwide. Munnoli et al. (2010) studied earthworm biodiversity, finding 44 species in the British Isles, 180 in France, 70 in Japan, 20 in Canada, 192 in New Zealand, 15 in Pakistan, 3 in Libya, 300 in Australia, and 509 in India. The species most frequently utilized in vermicomposting and vermiculture practices globally include *Eisenia andrei* and *Eisenia fetida* (Reynold, 1977). In India, common earthworm species such as *Eutyphoeus waltoni*, *Metaphire posthuma*, *Perionyx excavatus*, and *Lampito mauritii* are widely distributed across various habitats. Additionally, other genera, including *Amyntas*, *Metaphire*, *Drawida*, and *Eutyphoeus*, also encompass significant species. Commonly found Indian species consist of *Lumbricus terrestris*, *Lumbricus rubellus*, and *Eisenia fetida*. These earthworms play a significant role in maintaining soil health, as they facilitate nutrient cycling by decomposing organic matter and reintegrating vital nutrients into the soil. Identification of these species is typically based on their segmentation patterns and body coloration.

Earthworms act as exceptionally efficient instruments for environmental assessment.

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How to cite this article:

Momin, S., Momin, H., & Manna, N. (2025). Comparative assessment of occurrence of *Monocystis sp.* in wild and cultured varieties of *Lumbricus terrestris* (Linnaeus, 1758). *Royal International Global Journal of Advance and Applied Research*, 2(6), 26–31. <https://doi.org/10.5281/zenodo.17045743>

Theses vermiform environmental engineer have the capacity to bio-accumulate a range of heavy metals, industrial waste, herbicides, pesticides, and their byproducts (Edwards and Bohlen 1996; Gingleman et al.1998). They have inherent ability in the recycling and re-purposing of vital materials necessary for the existence of both flora and fauna (Lee and Foster 1991; Edward et al. 1995). Earthworms are considered as a master builder of top soil and by consuming organic matter, earthworms hasten the decomposition of materials into smaller, more manageable components that other organisms can utilize. Without this pivotal decomposition process, fundamental life-sustaining chemicals would remain locked away and unreachable to other life forms.

Additionally, earthworms accelerate plant growth by chemically and biologically altering the soil. They achieve this by mineralizing organic matter and promoting the activity of certain plant parasites and symbionts. Earthworms are helpful in transforming large quantities of organic material, such as decaying leaves, into nutrient-rich humus, thereby enhancing soil fertility. This change happens when earthworms drag organic material, like fallen leaves and manure, into their tunnels, either to devour or to shut off their paths. Once inside the burrow, they break down and partially digest the organic material, mingle it with soil by adding their intestinal fluids. The casts produced by earthworms contain approximately 40% more humus than the upper nine inches of the surrounding soil, along with dead organic matter. Additionally, earthworms ingest small soil particles, including stones as small as 1/20 of an inch (1.25 mm), which are ground into a fine paste in their gizzards before being digested in the stomach. The excretion of this processed material in the form of casts, which are deposited either on the surface or deeper within the soil, makes minerals and plant nutrients readily available. It is noteworthy that a significant proportion of earthworms may be affected by parasitic infections.

Monocystis species represents an apicomplexan parasite that resides within the seminal vesicle of terrestrial earthworms. The presence of this parasite is consistently observed in the seminal vesicle of infected earthworms. Infection occurs when an earthworm consumes a spore that harbors multiple sporozoites. These sporozoites hatch within the gizzard, subsequently penetrating the intestinal wall and entering the dorsal blood vessel, ultimately reaching one of the host's five or so hearts. From this location, they invade the seminal vesicle, where they infiltrate the sperm-forming cells in the vesicle wall, leading to the destruction of developing spermatocytes. The parasite derives nourishment from the sperm morula by secreting enzymes and absorbing the resultant digested materials through its pellicle. It often migrates to another morula to consume the cytoplasm before the morula reaches full maturity. This cytoplasmic consumption adversely impacts the reproductive capabilities of the earthworm. Following this phase, the parasites migrate into the lumen of the vesicle, maturing into trophozoites. After a feeding period, two trophozoites will merge, flatten against one another, and secrete a shared cyst, known as a gametocyst, which

typically contains two gamonts. Each gamont then undergoes significant nuclear division, pinching off a small portion of cytoplasm that buds off to form gametes. The fusion of two gametes results in the formation of a zygote, which subsequently develops into a spore. Following three rounds of cell division, 8 sporozoites are produced, leading to each gametocyte containing numerous oocysts (Schmidt and Robert 2000 and Bush et al. 2001). New hosts become infected through the ingestion of gametocytes or, more frequently, by consuming individual oocysts. Consequently, meiosis occurs at the zygotic stage, with only the zygote being diploid; the reductional division during sporogony restores the sporozoites to a haploid state.

Gregarines are specialized parasites that exhibit host specificity among a diverse array of invertebrate taxa, including those from the phyla Arthropoda (such as crustaceans, insects, and myriapods), Annelida (encompassing polychaetes, oligochaetes, and leeches), Nemertea, Mollusca, Echinodermata, and Urochordata. Their research is somewhat constrained, mainly because of their absence of connection with vertebrate hosts and the slight damage they cause to their invertebrate counterparts. Currently, there are around 1,600 recognized species, although many remain inadequately researched, suggesting the potential existence of numerous undiscovered species. Notable examples include *Monocystis lumbrici*, *Monocystis pheretemi*, *Monocystis beddardi*, *Monocystis magna*, *Monocystis agilis*, and *Monocystis bengalensis*. These organisms are monogenetic, with the decay of the host leading to the release of spores into the soil. These spores can be ingested by other worms, facilitating transmission to new hosts. Additionally, spores may be transferred during copulation, although they are not observed within cocoons. Sporocysts are excreted through bird feces and subsequently consumed by worms, which are then preyed upon by birds, perpetuating the cycle. In certain hosts, reproduction occurs within the coelomic fluid, with spores residing in the posterior segments. *Monocystis* does not adversely affect earthworms, nor does the host exhibit any significant impact from the parasites. Research indicates that there is no immediate detriment to growth or reproductive success in *Lumbricus terrestris* due to *Monocystis sp.* infection, and skin coloration may serve as an indicator of parasite load. The initiation of sexual activity is crucial in the context of parasitic infections. (Stuart Field et al., 2005). Furthermore, the abundance of available resources results in a lack of intra-specific competition among *Monocystis*.

The transmission mode of *Monocystis* is prevalent among earthworms and other oligochaetes. Research indicates that *Monocystis* does not adversely affect the reproductive viability or physiological health of earthworms (Field & Michiels, 2005). While the complete life cycle of *Monocystis* has been documented, the precise origins of this organism remain to be determined. In consideration of the aforementioned literature, the present study was undertaken.

India, being a major agrarian economy, earthworms serve as an indispensable asset. Rightly quoted as friends of farmers, and this age of organic farming. Vermicast left by earthworms can be used as a suitable feed

for poultry and even aquaculture. Parasitic infection of *Monocystis* sp. is a concerning matter for culturists who look forward to utilizing healthy worms and using them for agriculture or allied activities. This study addresses the gap of concern of infection in the same.

Materials & Methodology

Lumbricus terrestris were obtained from wild (Sanjay Gandhi National Park, Mumbai) during the monsoon period and cultured sources (Ramnarain Ruia Autonomous College Greenhouse Management department, Mumbai) and identified using a suitable key (Sims & Gerard, 1985). As *Monocystis* feeds on sperm present in seminal vesicles, only sexually mature and healthy-looking worms

were chosen by checking the clitellum and motility. 3 individuals from each wild and cultured site were selected and anatomized separately. Care was taken not to mix the worms. Worms were washed and narcotized by being kept on ice packs. Animals were fixed on dissecting wax trays (figure 1), and incisions were given on the 13th to 15th segments. The whitish milky fatty-looking seminal vesicles were carefully removed with fine tweezers (figure 2), which were teased in 2 ml normal saline (0.9%) with clean forceps and needle (figure 3). A milky white suspension was obtained, of which a smear was prepared on a clean, grease-free slide and observed under a light microscope without staining.

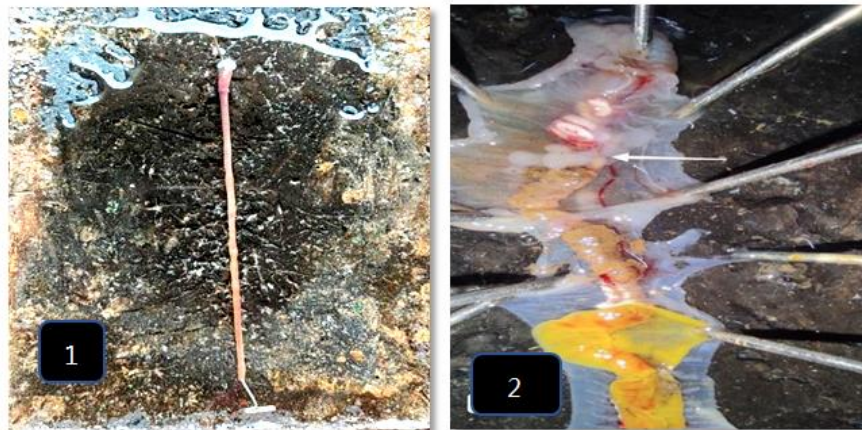


Figure 1: *Lumbricus terrestris* narcotized and pinned on dissecting tray.

Figure 2: Dissected Earthworm with arrow indicates seminal vesicles



Figure 3: Watchglass containing seminal vesicle suspension

Result and Discussion: No instances of *Monocystis* were detected in the cultured worms; however, a significant presence of earthworm sperm and sperm morulae was noted across the microscopic field in all three dissected cultured

earthworms (Figures 4, 5, and 6). In contrast, both the trophozoite and gametocyte stages of *Monocystis* were observed in large quantities in all three wild earthworms (Figures 7, 8, and 9).

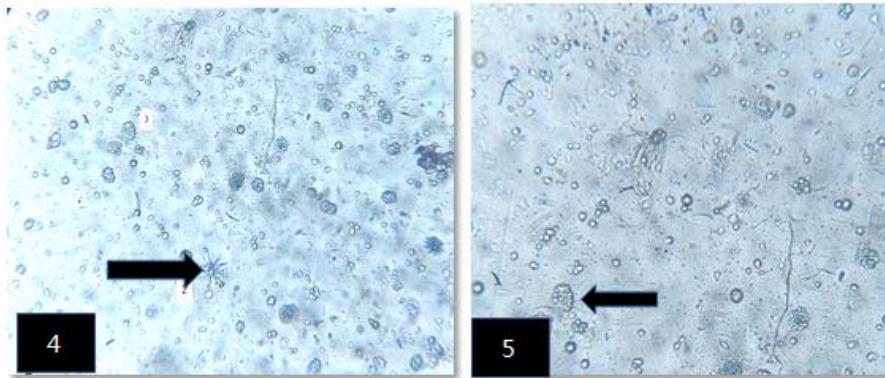


Figure 4: Arrow indicates sperm bundle (10X)

Figure 5: Arrow indicates sperm morula (10X)

Figure 6: Arrow indicates sperm bundle (10X)

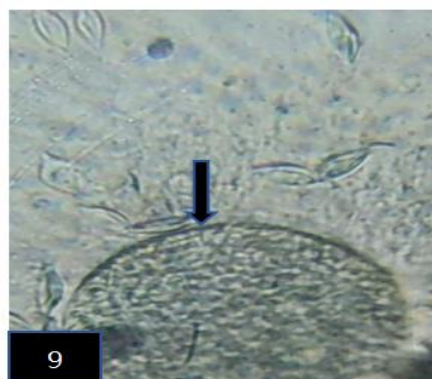
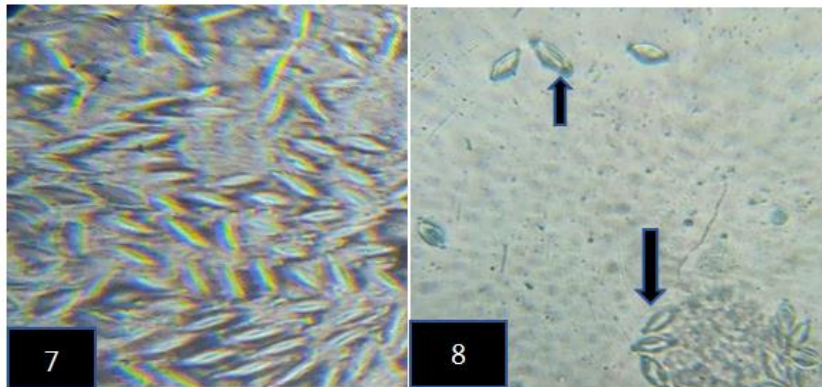


Figure 7: Abundant Trophozoite (10 X)

Figure 8: Arrow indicates Trophozoite (10X)

Figure 9: Arrow indicates Gamont (Gamatocyte) (10 X)



Our research indicates that wild populations of *Lumbricus terrestris* exhibit a higher susceptibility to *Monocystis* infection compared to their cultured counterparts. This heightened susceptibility can be linked to the presence of *Monocystis* spores in the soil, which arise from the death and decomposition of earthworms. As the host organism decomposes, spores are released into the soil, where other earthworms may consume them, helping in the spread of infection. Additionally, during mating, earthworms can transfer spores to each other, but these spores are not present in cocoons. The transfer of sporocysts occurs during mating or through the exchange of sperm from the seminal vesicle of one earthworm to another. Furthermore, sporocysts can be disseminated through the excreta of birds, which may inadvertently consume infected worms, perpetuating the cycle of infection (Keller Erin, 2016). The prevalence of this parasite is such that nearly all mature earthworms are likely to be infected. In contrast, earthworms raised in a vermiculture environment benefit from a more regulated habitat and a diet of organic material and soil that is periodically replenished, significantly reducing their risk of contracting *Monocystis* infection. Our findings could be further substantiated through more advanced methodologies and a greater number of anatomical examinations of earthworms.

The fully developed form of *Monocystis* is referred to as a trophozoite (Figures 7 & 8), which serves as the feeding stage. Initially, the young trophozoite resides within the sperm morula, a cluster of developing sperm cells in the host organism. During this phase, it derives nourishment from the protoplasm of the developing sperm, ultimately depleting it until it is encased by the tails of the deceased spermatozoa. At this point, it may be erroneously identified as a ciliated organism; however, the sperm tails eventually separate from the trophozoite, allowing it to become free. The adult organism exhibits an elongated, spindle-like, and flattened morphology, resembling a worm. Its surface is enveloped by a robust, smooth, and permeable pellicle. The cytoplasm is distinctly organized into ectoplasm and endoplasm, with the latter housing a prominent vesicular nucleus. The trophozoite's mode of nutrition is classified as sporozoic. Although it lacks specialized locomotory structures, it is capable of exhibiting wriggling and gliding motions.

Although it is observed that nearly all earthworms harbor *Monocystis* infections, these parasites do not significantly harm the earthworms (Keller Erin, 2016). While *Monocystis* does destroy the sperm of the earthworms, this does not compromise their fertility, as earthworms are capable of producing sperm in large quantities. Earthworms seem to defend themselves against these parasites by creating a resistant protective layer around the trophozoites and by eliminating the spores through innate process called phagocytosis. Moreover, the parasite has various adaptations that facilitates its survival and reproduction.

Conclusion:

The Lumbricid-*Monocystis* species model system is essential for studying host-parasite interactions. Of over 70 *Monocystis* species, only 15 are found in India. . Notably,

Monocystis odontotermi has been identified in the haemocoel of the termite *Odontotermes obesus*, which is a non-oligochaete host, while *Monocystis pontodrilus* has been found in the littoral oligochaete *Pontodrilus bermudensis*. The remaining *Monocystis* species have been associated with terrestrial earthworms, with no records from the current host, *Lumbricus terrestris*. The current study provides the initial definitive microscopic documentation of the presence of *Monocystis* in wild populations of *Lumbricus terrestris*, potentially identifying it as *Monocystis lumbrici*. Given that *Monocystis lumbrici* inhabits the seminal vesicles of *Lumbricus terrestris*, it is plausible that the observed *Monocystis* species may indeed be *Monocystis lumbrici*. However, further genetic analysis of the parasite is necessary to definitively identify it as *Monocystis lumbrici*, which predominantly impacts wild earthworms rather than those in cultivation. Our results could be reinforced through the application of more sophisticated techniques and an increased number of anatomical assessments of earthworms.

Acknowledgement

Authors are thankful to the principal and Head department of zoology, Ramnarain Ruia Autonomous College, Mumbai for providing necessary laboratory facilities to carried out this work.

Financial support and sponsorship

Nil.

Conflicts of interest

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

Authors' contributions: The experimental design was developed by Shakir Momin, while Nirmal Manna was responsible for conducting the experiment, generating the data. The manuscript was authored by Heena Momin.

Funding: The authors have stated that they did not receive any financial support from any source for this work.

Competing interests: The author's stated that the authors possess no competing interests.

Ethical approval and consent to participate: NA

Animal Ethics Statement: The ethics committee of Ramnarain Ruia Autonomous College, Mumbai evaluated the experimental protocol and concluded the ethical oversight was unnecessary.

Consent for publication: We affirm that this research is intended for publication in your journal. Additionally, I confirm that this paper has neither been published nor submitted for publication elsewhere. All the author's have approved the manuscript and agreed with submission to the journal.

Availability of data and material: All the obtained data in the present work are reported in this published article.

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