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Scanning electron microscopy based morphological characterization and prevalence of microsporidia in hymenopteran insects

Mukta Mayee Kumbhar[1*], Suman Mishra[2]
[1] Department of Life Sciences, Rama Devi Women's University, Bhubaneswar, Odisha, India
[2] Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India
Corresponding Author: Mukta Mayee Kumbhar
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Abstract
Microsporidia are considered to be highly diverse and ubiquitous intracellular parasites in the environment. They are known to infect almost all animal phyla but most prevalent in fish and arthropods. Among the insect orders, the order hymenoptera includes insects which are indispensable for pollination, biological control, and ecosystem functioning. However, they are infected by a number of fungal pathogens particularly microsporidia, that substantially reduce these benefits, essential for agricultural productivity, apiculture, and environmental health. The present study was conducted to investigate the prevalence rate of microsporidian infection in hymenopteran insects. Also morphological characterization of the microsporidia was done by light and scanning electron microscopy. The scanning electron microscopy revealed heavy infection of microsporidia with different development stages in gut tissue of insects. The spores were oval in shape whereas meronts were spherical in shape. In addition to this, the spore provided with rough surface with an average length ranging from 2 µm - 6 µm long and width ranging from 2 µm - 4 µm. In this investigation, a total of 500 insects from four families of order hymenoptera *viz*. Apidae, Vespidae, Scoliidae, Formicidae were collected and screened for the presence of microsporidian infection, out of which 187 insects were found positive with the infection. Hence, the overall prevalence of microsporidian parasites was 37.4%, indicating that microsporidian infections are common among hymenopteran insect populations.

Keywords: Arthropods, hymenoptera, microsporidia, pollination, apiculture

Introduction

Hymenoptera is an order in the class insecta of phylum arthropoda which contribute immensely to the environment particularly in the terrestrial ecosystem. The hymenopteran insect comprise of about 150000 described species including honey bees, sawflies, wasps, ants and represent one of the most diverse and ecologically important insect orders. These insects provide essential ecosystem services such as pollination, biological control of pests, seed dispersal, and nutrient cycling. Despite their ecological and economic importance, hymenopterans are susceptible to a wide range of pathogens, including viruses, bacteria, fungi, protozoa, nematodes, and microsporidia. Pathogen infections can adversely affect individual fitness, colony health, reproductive success, and population stability, thereby influencing ecosystem functioning. Among the intracellular pathogens, microsporidia have gained increasing attention because of their widespread occurrence and significant impact on hymenopteran hosts (Kumbhar and Mishra, 2016) [9]. Microsporidia are obligate intracellular fungal-related parasites that infect numerous invertebrate and vertebrate species. In Hymenoptera, species such as *Nosema ceranae*, *Nosema apis*, and *Vairimorpha* infect the epithelial cells of the insect midgut, causing energetic stress, tissue degeneration, impaired digestion, reduced longevity, decreased reproductive performance, and weakened immune responses. In eusocial insects such as honey bees and bumble bees, pathogen transmission occurs through contaminated food, feces, and close social interactions, facilitating rapid dissemination throughout colonies (Williams *et al*., 2014) [15]. Further, in order to maintain agriculture and biodiversity, hymenopteran pollinators are essential. Their significance is highlighted by their contribution to crop quality, ecological stability, and yield improvement. However, their population decreases and sometimes colony become collapsed due to many factors like pathogen infection, habitat loss, and climate change. Specifically, the lifestyles and feeding ecologies of some hymenopteran taxa render them especially vulnerable to pathogen infestation (Shoemaker, 2023) [12]. Co-infections involving viruses, fungi, and microsporidia may exacerbate disease progression and increase host mortality (Kumbhar *et al*., 2017a) [5].

Materials and methods

Collection and screening of hymenopteran insects for the presence of microsporidian infection

Hymenopteran insects were collected from the flower garden with the help of insect collecting net. They were kept in the insect collection box, brought to the laboratory and stored at 4°C for further screening. The insects were identified using a standard insect identification key (Soulsby EJL., 1982) [13]. The insects were homogenized individually in distilled water and filtered through Whatman filter paper. The filtrate was centrifuged at 8000 rpm for 30 min and the pellet was suspended in distilled water and the process was repeated three times by adding sterilized distilled water to the sediment. The final sediment obtained was suspended in minimal volume of sterilized distilled water and stored at 4° C (Kumbhar and Mishra, 2022) [8].

Prevalence rate (%) of microsporidian infection in hymenopteran insects

The prevalence percentage of microsporidian infection in insects were calculated by the following formula:

$$\text{Infection rate of microsporidia (\%)} = \frac{\text{No. of infected insects}}{\text{Total no. of collected insects}} \times 100$$

Light Microscopy of fresh microsporidia spores isolated from hymenopteran insects

The infection of microsporidia in hymenopteran insects were determined by examining homogenate smear under a bright field microscope at 400X magnification (Chakrabarty *et al*., 2013) [2]. The spores were photographed using an imaging microscope (EVOS XL system, 40X).

Scanning Electron Microscopy (SEM) of insect gut infected with microsporidia

For the SEM study, a small piece of the insect gut infected with microsporidian spores were fixed in 2.5% (v/v) glutaraldehyde ($C_5H_8O_2$) in 0.1 M phosphate buffer, pH 7.4 for 24 hours. Secondary Fixation of the sample was done in 1% osmium tetroxide (OsO_4), pH 7.4 for 1 hour followed by dehydration in increasing gradation of 30%, 50%, 70%, 90% and 100% acetone (Undeen, 1997) [14]. The dried sample was then mounted on copper stub using carbon tape and scanned under a JEOL JSM-6490LV scanning electron microscope, and the sizes of the spores were measured. SEM study was done at the University Science Instrumentation Centre (USIC), Babasaheb Bhimrao Ambedkar University, Lucknow.

Results and Discussion

In the present investigation, a total of 500 insects from four families of order hymenoptera *viz*. Apidae, Vespidae, Scoliidae, Formicidae were collected and screened for the presence of microsporidian infection, out of which 187 insects were found positive with the infection. Hence, the overall prevalence of microsporidian parasites was found to be 37.4%. Our study showed that microsporidian infection was prevalent in hymenopteran insects, with a prevalence variation from 13% to 78% of the colonies. Among all the insects studied, the highest prevalence percentage of microsporidian infection was recorded in *Apis mellifera* (78.33%, 94/120) followed by *Xylocopa violacea* (36%, 18/50), *Polistes flavus* (31.57%, 30/95), *Dorylus helvolus* (26.66%, 24/90), *Scolia soror* (15.38%, 10/65), *Lasius niger* (13.75%, 11/80) (Figure 1, Table 1). This result is supported by a previous study of Lotfi *et al*. (2009) [11] who reported highest infection rate of *Nosema* infection *in A. mellifera* in May (83.3%) whereas 59.5% of *Nosema* infection was recorded during spring season and a minimum of 3.3% of infection was observed in summer season. In other reports, the microsporidian infection in honey bee was generally higher in the rainy season than in the dry season, with the highest prevalence of 71.4 % during the months of August (Dawet *et al*., 2016) [3]. In a study, the prevalence rate of microsporidian infection in aphids were found to be 30.46 % (Kumbhar, 2026) [10]. A very low percentage of microsporidian infection was recorded in *Danaus genutia* (Striped tiger butterfly) and *Melanitis leda leda* as 11.11% and 6 % respectively (Kumbhar and Mishra, 2022) [8]. Similarly, among the 180 Fire Ant, *Solenopsis daguerrei* individuals collected, seven ants (3.9%) were tested positive for microsporidian *Kneallhazia solenopsae* infection (Ascunce *et al*., 2023) [1]. These differences in the prevalence rates of microsporidia among insect hosts clearly indicate that the prevalence of microsporidian infections varies according to both climatic conditions and host species (Kumbhar *et al*., 2017b) [6]. Further, the study concluded that nosemosis disease in *A. mellifera* reduced worker longevity by 22-44% which in turn reduced the honey production, therefore causing heavy economic losses (Higes *et al*., 2010) [4]. In case of severe infection of nosemosis in honey bee, it lead to Colony Collapse Disorder and thereby greatly affecting the economy of a country because a single infected honey bee can be responsible for the rapid transmission of disease in the colony (Kumbhar and Mishra, 2024) [7]. Under the light microscope, the microsporidian spores isolated from hymenopteran insects were observed as translucent round/oval spores with characteristics brownian movement. The scanning electron microscopy revealed heavy infection of microsporidia with different development stages *viz*. mature spore stage (S), meront (M), and dividing spores by binary fission inside the gut tissue of insect. Few microsporidia were seen proliferating from the gut tissue (Figure 2, Table 2). The spores were oval in shape whereas meronts were spherical in shape. In addition to this, the spore provided with rough surface with an average length ranging from 2 µm - 6 µm long and width ranging from 2 µm - 4 µm. Some of the clustered spores were also observed under SEM study. From the present study, it is evident that microsporidian parasites are common pathogen of hymenopteran insect.

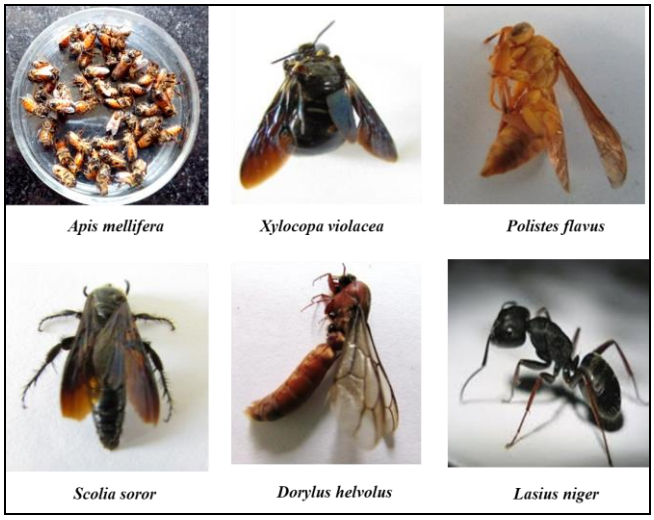

Fig 1: Photographs of the collected hymenopteran insects

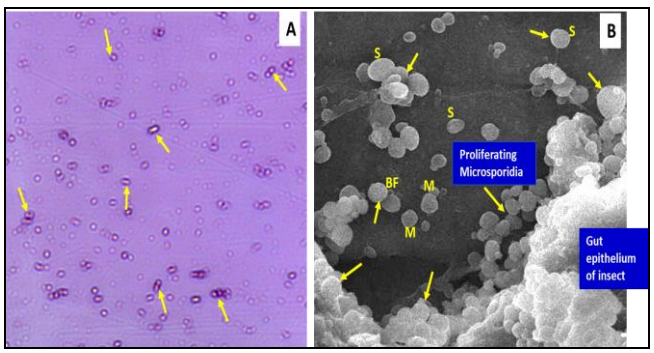

Fig 2: Arrow indicating the presence of microsporidian spores A) Light Micrograph of microsporidia isolated from honeybee at 400X magnification B) Scanning electron micrograph showing extensive microsporidian infection in the gut epithelium of a honey bee with different developmental stages: Spore (S), Meront (M), Spore Dividing by binary fission (BF)

Table 1: Morphological Characterization of Microsporidia Isolated from Hymenopteran Insects Using Scanning Electron Microscopy (SEM)

Characterization of Microsporidia	Observation
Spore shape	Spherical, Oval
Surface	slightly rough
Size	2–6 µm long and 2-4 width
Arrangement	Individual or clustered spores
Host tissue	heavy spore accumulation in gut tissue
Infection intensity	Dense spore masses in severely infected tissues

Table 2: Prevalence rate (%) of microsporidian infection in hymenopteran insects

Phylum- Arthropoda Class- Insecta Order- Hymenoptera					
Family	**Insect species**	**Common Name**	**No. of specimen collected**	**No. of Specimen infected**	**Prevalence rate (%)**
Apidae	*Apis mellifera* (Linnaeus, 1758)	Western honeybee	120	94	78.33
	Xylocopa violacea (Linnaeus,1758)	European Carpenter Bee	50	18	36
Vespidae	*Polistes flavus* (Cresson, 1868)	Yellow jacket wasp	95	30	31.57
Scoliidae	*Scolia soror* (Smith, 1855)	hairy flower wasp	65	10	15.38
Formicidae	*Dorylus helvolus* (Linnaeus, 1764)	Driver ant	90	24	26.66
	Lasius niger (Linnaeus, 1758)	Black Garden Ant	80	11	13.75
	Total		500	187	37.4

Conclusion

The present investigation demonstrated the occurrence of microsporidia in hymenopteran insects, indicating that these parasites are present within the surveyed populations. This study provides brief information about the morphology and ultrastructure and prevalence of microsporidian parasites harbouring in hymenopteran insect population. The observed prevalence provides valuable baseline information on the distribution of microsporidian infections in hymenopteran hosts. These findings contribute to a better understanding of host–parasite interactions and highlight the need for further investigations to identify the microsporidian species involved, assess their pathogenicity, and evaluate their ecological and economic significance.

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