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Prevalence and diversity of entomopathogenic microsporidian parasites in lepidopteran insects

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Abstract
The Microsporidia is a diverse phylum of eukaryotic endoparasite infecting host groups from all major taxa in all environments ranging from those infecting pest and beneficial insects, to important parasites of humans. Microsporidian infections in insects are generally chronic, causing pathogenic effect on host and reduce their fecundity and life spans. As microsporidia are emerging pathogen, it is important to know their diversity associated with Lepidopteran insects. In the present investigation, a total of 13 different species of butterfly and moths were collected from the insect collection sites. The insects belonging to order Lepidoptera were again categorized into 6 families *viz.* Nymphalidae, Pieridae, Papilionidae, Saturniidae, Sphingidae and Erebidae. Among collected Lepidopteran insects, highest prevalence of microsporidian infection was reported in *Danaus chrysippus* (33.51%) whereas least prevalence was reported in *Melanitis leda leda* (6%). Therefore, the present study surely gives a brief idea about the distribution and prevalence percentage of microsporidian infection in the lepidopteran insect population commonly inhabiting the agricultural and floral fields.

Keywords: Insects, lepidoptera, endoparasite, diversity, emerging pathogen

Introduction
The Microsporidia are a group of eukaryotic intracellular parasites, first recognized in 1857 with the description of *Nosema bombycis* Nägeli as a parasite of silkworms (Wittner and Weiss 1999). The parasite belongs to the phylum microsporidia which include almost 1,500 species belongs to 200 genera (Vávra and Lukeš 2013) [20]. They are ubiquitous in the environment and known to infect a wide range of vertebrate and invertebrate hosts including insects, ciliates, molluscs, crustacean, fish, birds, dog, cat, rabbits and human (Canning and Lom 1986; Snowden and Shadduck 1999) [6, 17] but most prevalent in insects and fish (Becnel and Andreadis 1999). Sometimes, they have disastrous effect on various industries *viz.* apiculture, sericulture and aquaculture industries leading to enormous loss (Dunn and Smith 2001; Dunn *et al*. 2001) [8, 9]. The infective stage of microsporidia in the spore includes a highly developed injection apparatus called polar tube that penetrate the host cell and inject the infective sporoplasm into host cytoplasm and this process takes less than 2s *in- vitro* (Frixione *et al*. 1992) [12]. These pathogens then undergo most of their development within the host cytoplasm, which allows direct transport of nutrients and energy from the host to the pathogen.

Materials and Methods
Collection of Lepidopteran insects
The lepidopteran insects were collected from floral and vegetation garden. The insects were collected by standard insect collection techniques (Fenemore and Prakash, 1992) [11] and brought to the laboratory for screening and isolation of microsporidia. The Lepidopteran insects were collected with the help of an insect collection net (with a light and strong handle of 1 m length, rim of 0.3 m diameter made of heavy wire and a nylon net bag with open mesh). After collection, the insects were transferred immediately to plastic boxes with perforated lids and bought to the laboratory for further identification and examination for microsporidian infection.

Identification of collected insect pests
The collected insects were identified with the help of literature surveys and online insect identification guide (Gasse, 2013; BugGuide) [13].

Screening of Insect Pests for Microsporidian infection
Homogenization
The abdomen of each insect was separated and homogenized individually in 0.6% K2CO3 (4ml/g) solution and investigated for the presence of microsporidian spores.

Microscopic examination
The smear was prepared from the respective homogenate and observed for the presence of microsporidian spores under light microscope at 400X (Figure 2). If the samples were found negative for the microsporidian infection,

the samples were discarded whereas in case of positive infection the samples were stored at 4°C for further processing and studies.

Prevalence of microsporidian infection in Insects

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

$$\text{Prevalence \% of Microsporidia} = \frac{\text{No. of infected insect} \times 100}{\text{Total no. of collected insect}}$$

Results

In this study, an attempt was made to investigate the occurrence and prevalence of microsporidia in lepidopteran insects that are commonly available in the vegetation and floral garden. The Lepidopteran insects collected were categorized into 6 families *viz*. Nymphalidae, Pieridae, Papilionidae, Saturniidae, Sphingidae and Erebidae. The collected butterflies belonging to Nymphalidae family were *D. chrysippus* (Plain tiger butterfly), *D. genutia* (Striped tiger butterfly), *M. leda leda* (Common Evening brown butterfly), *M. phedima* (Dark evening brown butterfly) *and J. almana* (Peacock pancy butterfly). Among these, all the insects were found positive with the microsporidian infection except *J. almanac* (Figure 1). The plain tiger butterflies were most commonly available. In the present study a total of 185 plain tiger butterflies were collected, out of which 62 were found to be infected with the microsporidia; therefore the prevalence percentage of the microsporidian infection in the respective butterfly was found to be 33.51 %. A total of 27 *D. genutia*, 20 *M. leda leda* and 79 *M. phedima* butterflies were collected, out of which 3, 1 and 20 butterfly specimens in the respective group were found infected with microsporidian infection. Therefore, the prevalence of microsporidian infection in *D. genutia* (Striped tiger butterfly), *M. leda leda* and *M. phedima* was calculated as 11.11%, 6 % and 25.31 % respectively (Table 1). Four different species of butterflies' *viz*. *C. florella, C. Pomona, C. pyranthe* and *E. brigitta* were collected that belonged to the family Pieridae. Among these, microsporidian infection was not detected in the butterfly *C. Pomona* and *E. brigitta* where as positive microsporidian infection was observed in the butterfly *C. florella* and *C. pyranthe*. A total of 86 *C. florella* butterflies and 36 *Catopsilia pyranthe* butterflies were collected, out of which 21 *C. florella* and 5 C. pyranthe butterflies were found to be infected with the microsporidian infection. Hence, the prevalence percentage of microsporidian infection in *C. florella* and 36 *C. pyranthe* butterflies were calculated as 24.42% and 13.88% respectively. A total of 23 lime swallowtail butterflies (*P. demoleus*) were collected from Family Papilionidae and all were found to be negative for the microsporidian infection. A total of 52 Indian eri silkworms (*S. cynthia ricini*) were collected from Saturniidae Family, out of which 17 were found infected with microsporidia. So, the prevalence of microsporidian infection in Eri silkworms was calculated as 32.69%. A total of 16 lesser death's head hawk moth (*A. styx*) were collected Sphingidae family and all were found negative with the microsporidian infection. A total of 68 common grass moths (*Caenurgina erechtea*) were collected family Erebidae and all were found negative with the microsporidian infection.

Discussion

Among the entomopathogens, microsporidia are highly ecologically diverse parasites that have gained prominence both as pathogens and potential biocontrol agents. They also represent a model system for research on host-parasite interactions which is the least studied group as far as their utilization for biological control of noxious insect pests is concerned. However, the infections often go unnoticed and their role in population dynamics of insects is often not recognized, because microsporidia do not cause dramatic epizootics such as those caused by bacteria and viruses. There are few literatures available about the prevalence of microsporidian parasites in insects. The results of the present study showed that all butterfly species inhabiting the surveyed areas had microsporidian infection except the Peacock pancy butterfly, *J. almana*; whereas infection was not found in the two moth species investigated i.e. Lesser death's head hawk moth, *A. styx* and Common Grass moth, *C. erechtea*. Studies by several workers have also indicated that microsporidian infection is very common in Lepidopteran insects (Kishore *et al*., 1994; Choi *et al*., 2002; Singh *et al*., 2008; Bashir *et al*., 2011; Bhubaneswari and Surendra Nath 2015a, 2015b) [14, 7]. In the butterflies and moth species investigated in the present study the maximum prevalence percentage of microsporidian infection was recorded in *D. chrysippus* (33.51%) followed by *S. cynthia ricini* (32.69%), *M. phedima* (25.31%), *C. florella* (24.42%), *C. pyranthe* (13.88%), *D. genutia* (11.11%), and *M. leda leda* (6%). The result of the present study is more or less supported by the previous study by Bashir *et al*. (2011) where they have screened the lepidopteran insect pests of mulberry and agricultural crops collected from in and around Mysore, Karnataka, India and reported the prevalence percentage of microsporidian infection as 25.60%, 25.33%, 20.00%, 19.31% and 18.40% respectively in insect species *Spilosoma obliqua, Catopsilia pyranthe, Pieris rapae, Diaphania pulverulentalis*, and *Catopsilia crocale*. These authors have also reported that some of the lepidopteran insects were negative for microsporidian infections *viz*. *Sesamia inferens, Phytomyza atricornis, Eupterote mollifera, Terias hecabe, Laphygma exigua* and *Colias eurytheme*. Eveleigh *et al*. (2012) [10] also reported that, the annual spruce budworm (*Choristoneura*

fumiferana) mortality associated with microsporidium *N. fumiferanae* was ≤15% of all mortality in reared specimens and was positively correlated with but generally less than 30% of annual *N. fumiferanae* prevalence.
Earlier studies have reported the occurrence of microsporidian infection in the butterflies *C. pomona*, *P. demoleus* and *Eurema* sp. (Smitha, 2011) [16], however in the present investigation, the microsporidian infection was not detected in these butterflies. It may be due to the fact that microsporidian infections vary from one geographical climatic condition to other. Solter *et al*. (1997) [18] reported five biotypes of microsporidia in European populations of *Lymantria dispar* but none were found in North American population of *L. dispar*. In the present study, microsporidian infection was not found in the peacock pancy butterfly, *J. almanac*. A similar report was also given by Smitha (2011) [16] wherein she reported that the peacock pancy butterfly, *J. almanac* was free of microsporidian infection.

Conclusion

Microsporidia are diversely distributed, opportunistic, and density-dependent parasites infecting hosts of both invertebrate and vertebrate phyla including humans and assumed to be ubiquitous in the environment. It is also reported that that the microsporidian species that infect insects have been found in human, possibly transmitted through the insect bites, skin lesions, or contaminated food and water. In the present study, highest prevalence percentage of microsporidian infection was recorded *D. chrysippus* (33.51%) followed by *S. cynthia ricini* (32.69%), *M. phedima* (25.31%) and *C. florella* (24.42) which showed the microsporidian infection is consistent and common in lepidopteran insects. As microsporidia are emerging pathogen, a broad research investigation will provide an insight of the prevalence and diversity of these parasites in other unexplored lepidopteran insects. Also, the diversity and host range data of parasites can be used to evaluate their prevalence in lepidopteran insect populations. Host specificity studies contribute to our understanding of the evolutionary adaptability of microsporidia to new hosts.

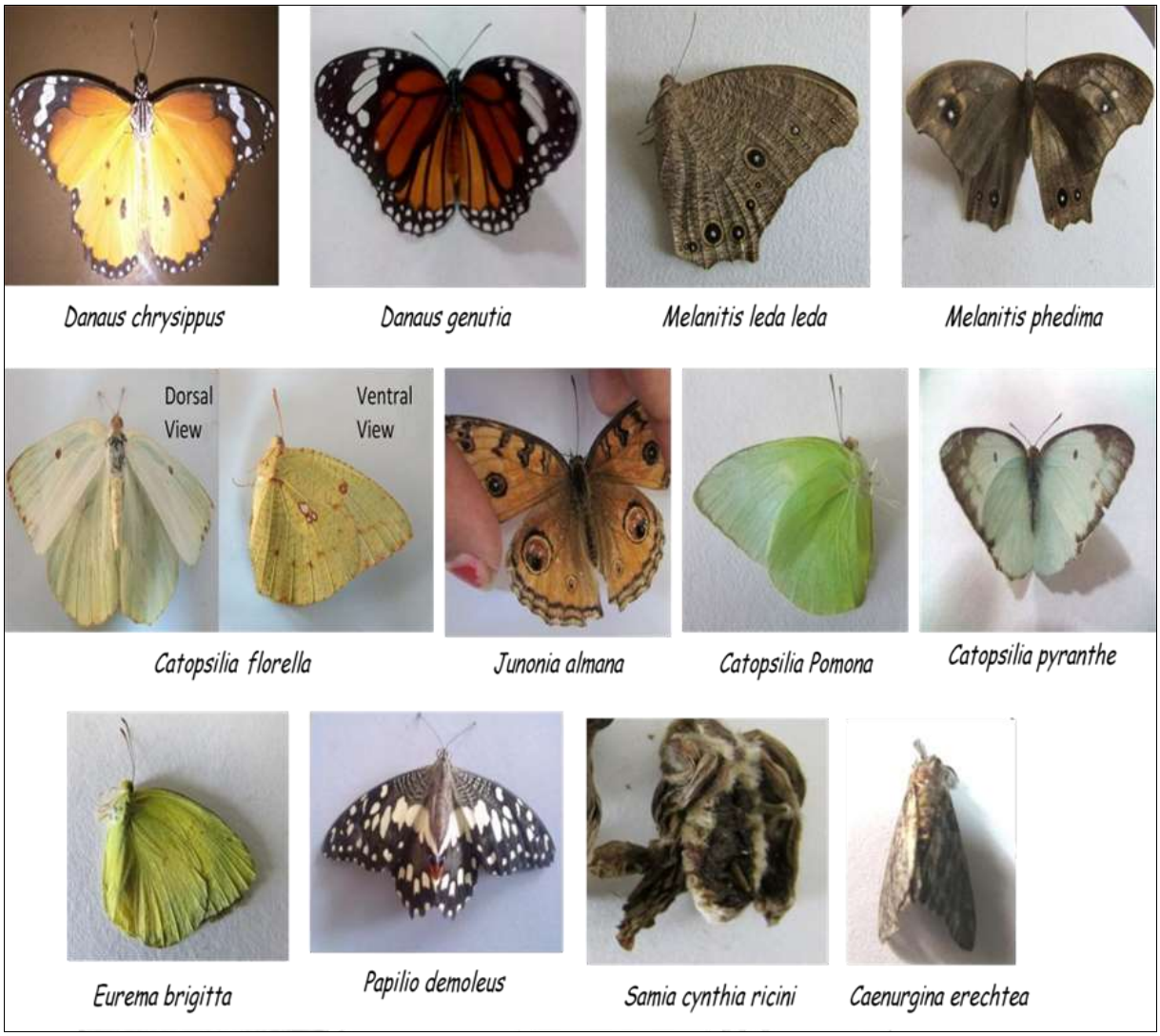

Fig 1: Photographs of the collected lepidopteran insects

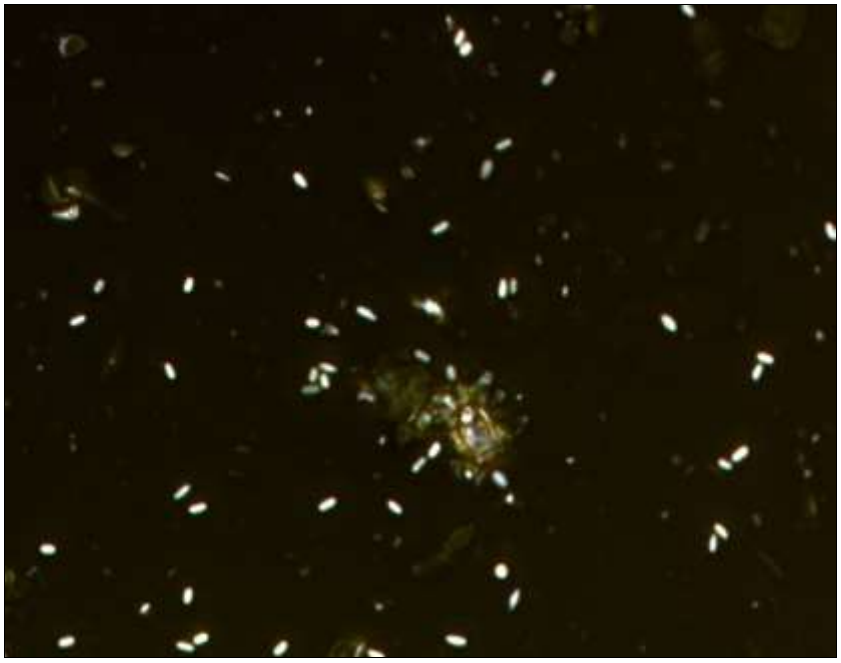

Fig 2: Light Micrograph of Microsporidia isolated from *D. chrysippus*

Table 1: Prevalence of microsporidian infection in insect species

Sl No	Insect species	Common Name	Family	No. of Specimen collected	No. of infected specimen	Prevalence %
	Danaus chrysippus (Linnaeus, 1758)	Plain tiger butterfly	Nymphalidae	185	62	33.51
	Danaus genutia (Cramer 1779)	Striped tiger butterfly	Nymphalidae	27	3	11.11
	Melanitis leda leda (Linnaeus, 1758)	Common Evening Brown butterfly	Nymphalidae	20	1	6.00
	Melanitis phedima (Cramer, 1780)	Dark Evening Brown butterfly	Nymphalidae	79	20	25.31
	Junonia almana (Linnaeus, 1758)	Peacock pancy butterfly	Nymphalidae	26	0	0
	Catopsilia florella (Fabricius, 1775)	African emigrant butterfly	Pieridae	86	21	24.42
	Catopsilia pomona (Fabricius, 1775)	Lemon emigrant butterfly	Pieridae	20	0	0
	Catopsilia pyranthe (Linnaeus, 1758)	Mottled emigrant butterfly	Pieridae	36	5	13.88
	Eurema brigitta (Cramer, 1780)	Small grass yellow butterfly	Pieridae	18	0	0
	Papilio demoleus (Linnaeus, 1758)	Lime swallowtail butterfly	Papilionidae	23	0	0
	Samia cynthia ricini (Drury, 1773)	Indian eri silkworm	Saturniidae	52	17	32.69
	Acherontia styx (Westwood, 1847)	Lesser death's head hawk moth	Sphingidae	16	0	0
	Caenurgina erechtea (Cramer, 1780)	Common Grass Moth	Erebidae	68	0	0

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