Asian Journal of Biological and Life Sciences | Original Research

Record of microspodian parasitisation of dung beetles (Coleoptera:Scarabidae) in a natural scrub ecosystem of Tamil Nadu

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Submitted : 20.08.2013 Accepted : 13.09.2013 Published : 31.12.2013

Abstract

The two year survey of microsporidia parasitizing dung beetles in a natural scrub jungle ecosystem of Tamil Nadu indicated twelve of the thirty three species of dung beetles to be infected with *Nosema* sp. whereas five species were found to be infected with *Vairimorpha* sp and mixed infection was observed in three species of *Onthophagus* beetles. The percentage of infection is very low in *Copris excisus* (2.5%) and high in *Euoniticelles fulvus (63.75%),* the spore yield was high in *Oniticellus cinctus (3.4 x10^6)* among the dung beetle populations of the scrub jungle. Being essentially nature's scavengers, the role of microsporidians in limiting the ecological services of the dung beetles is discussed.

INTRODUCTION

The super family Scarabaeoidea is one of the largest subdivisions of beetles with an estimated 35,000 species distributed worldwide. Dung beetles in the family's Scarabaeidae and Geotrupidae aid in the decomposition of dung, providing many benefits to pasture and animal health [1]. They compete with pestiferous flies and parasitic nematodes for dung resources, enrich the soil by burying large quantities of nutrient-rich dung, and effectively mix and aerate soil through tunnelling. Availability of Mammalian dung is the major factor that limits the distribution of populations of dung beetles and parasitic infection appears to control their abundance. Among the parasites, the protozoan microsporidians have been recorded to infect dung beetles [2]. Microsporidia infect wide range of vertebrate and invertebrate taxa. More than 250 species of microsporidia have been described from insects which represent only a small fraction of the total number of species present in insects [3]. Currently almost half of the described genera of microsporidia have insect as the host [4]. An attempt has been made here to survey the microsporidian infection of dung beetles in the natural scrub jungle ecosystem to provide base line data on the percentage infection of microsporidia in different species of dung beetles inhabiting the area.

MATERIALS AND METHODS

Dung beetles being commonly associated with dung, the dung pads were randomly selected from natural scrub jungle ecosystem. Beetles were collected from fresh dung using water flotation method and hand picking method. All species encountered were enumerated both in terms of the numbers of species as well as the number of individuals of each species. The beetles were brought to the laboratory and their gut dissected in ice cold insect saline. In total, 75 individuals of each species was sacrificed to obtained data on the percentage of infection.

Individual beetle was homogenized in a small volume of sterile water and the homogenate examined under WILD M3Z HEERBUGG binocular microscope. The homogenate from several infected specimens were pooled together and filtered through two layers of moist cheese cloth to remove body tissues. The filtrate was centrifuged at low speed for one minute to remove larger tissue particles. The supernatant liquid which contained majority of the spores was collected and centrifuged at 3000 rpm for 5 minutes to sediment the spores. The bottom portion which appeared as a whitish precipitate containing mainly spores was dispersed in a small volume of sterile distilled water and centrifuged again at 3000rpm for 10 minutes and the procedure was repeated three to four times for purifying the spores further. The concentrated sediment was centrifuged through 50% percoll in sterile distilled water containing 0.01M ammonium chloride (to avoid spore germination) at 750g for 20minutes. The spores that settled at the bottom were collected, washed with sterile distilled water to remove the percoll and stored at 4°C with the addition of 5g streptomycin per ml suspension until used [5]. For light microscopy, spores were stained with Giemsa staining. The length and breadth of the spores was measured using an ocular micrometer.

RESULTS

Spores of Microsporidia were identified by their characteristic shape, lustre and Brownian movement and were easily distinguished from other bacterial or fungal spores. Morphological features of spores were recorded by observing them under phase contrast microscope. The mean size of the spores was recorded (Table 1). The spores of *Nosema* sp. were ovo-cylindrical in shape and diplokaryotic whereas the spores of *Vairimorpha* sp. were found to be spindle shaped and binucleated. Of the 33 dung beetle species screened, twenty were infected with microsporidia. *Copris signatus*, *Copris excisus*, *Copris minutus, Caccobius vulcanus Tiniocellus spinipes, Gymnopleurus koenigi, Gymnopleurus gemmatus, Euoniticelles pallipes, Euoniticelles fulvus, Euoniticelles pallens, Sisyphus crispatus,*and *Sisyphus neglectus* are the species of dung beetles there were infected with *Nosema sp.. Oniticellus cinctus, Digitonthophagus bonasus, Digitonthophagus gazelle, Aphodius conspurcatus,* and *Aphodius prodromus* were found to be infected with *Vairimorpha sp.* whereas the three *Onthophagus sp.* showed mixed infection of both *Nosema* and *Vairimorpha.* The highest percentage of infection was recorded in *Euoniticelles fluvus (63.75)* although their percentage abundance in the ecosystem was low (4.599%). The least percentage of infection was observed in *Copris excises (2.5%).* Even though *Aphodius conspurcatus* was the most

Table 1. Length and width (Mean µm±SE) of microsporidian samples using ocular micrometer

S.No	Host name	Microsporidia	Length	Width	Percent infection	Spore load
1	*Aphodius conspurcatus*	*Vairimorpha* Sp.	4.7 ± 0.15	2.8 ± 0.13	40	$2.4x\ 10^4$
2	*Aphodius prodromus*	*Vairimorpha* Sp.	4.9 = 0.1	2.2 ± 0.13	46.25	$2.1x10^5$
3	*Copris signatus*	*Nosema* Sp.	4.3 ± 0.15	2.6 ± 0.16	12.5	$3.2x10^3$
4	*Copris excisus*	*Nosema* Sp.	3.9 = 0.1	2.1 ± 0.11	2.5	$1x10^2$
5	*Copris minutus*	*Nosema* Sp.	4.1± 0.24	2.3± 0.17	31.25	$2.7x10^4$
6	*Caccobius vulcanus*	*Nosema* Sp.	4.7 ± 0.15	2.9 ± 0.1	13.75	$1x10^3$
7	*Digitonthophagus bonasus*	*Vairimorpha* Sp.	5.6 ± 0.22	3.1 ± 0.1	18.75	$3.4x10^5$
8	*Digitonthophagus gazella*	*Vairimorpha* Sp.	4.9 = 0.1	2.9 ±0.1	17.5	$1.8x10^3$
9	*Euoniticelles pallipes*	*Nosema* Sp.	3.4±0.15	2±0.1	55	$2.2x10^4$
10	*Euoniticelles fulvus*	*Nosema* Sp.	3±0.21	0.9± 0.21	63.75	$2x10^4$
11	*Euoniticelles pallens*	*Nosema* Sp.	3.2±0.17	1.3±0.15	47.5	$3.2x10^3$
12	*Gymnopleurus koenigi*	*Nosema* Sp.	2.9 ± 0.17	1.5 ± 0.16	38.75	$2.4x10^2$
13	*Gymnopleurus gemmatus*	*Nosema* Sp.	2.3 ± 0.17	1.1 ± 0.16	33.75	$2x10^3$
14	*Oniticellus cinctus*	*Vairimorpha* Sp.	4.8 ± 0.13	3 ± 0.1	25	$3.4x10^6$
15	*Onthophagus centricornis*	*Vairimorpha* Sp. & *Nosema* Sp.	4.6 ± 0.16 3.3± 0.54	2.8 ± 0.13 2.3± 0.17	43.75	$2.4x10^4$ $1.7\ x10^2$
16	*Onthophagus tragoides*	*Vairimorpha* Sp. & *Nosema* Sp.	4.9 = 0.1 3.1 ± 0.24	3 ± 0.1 0.9± 0.21	23.75	$3.2x10^5$ $2.2x10^4$
17	*Onthophagus cervus*	*Vairimorpha* Sp. & *Nosema* Sp.	4.1 = 0.1 2.9± 0.15	2.9 ± 0.13 1.1 ± 0.16	50	$2.4x10^3$ $2x10^2$
18	*Sisyphus crispatus*	*Nosema* Sp.	2.9± 0.15	1.4±0.21	16.25	$1.4x10^3$
19	*Sisyphus neglectus*	*Nosema* Sp.	2.5±0.1	0.9±0.15	20	$1x10^3$
20	*Tiniocellus spinipes*	*Nosema* Sp.	3.6±0.22	1.3±0.13	58.75	$2.4x10^4$

Table 2. Relative Abundance of different species of dung beetles in the Natural Scrub jungle ecosystem.

S.No	Host name	Relative abundance (%)
1	*Aphodius conspurcatus*	21.599
2	*Aphodius prodromus*	11.659
3	*Copris signatus*	2.343
4	*Copris excisus*	1.162
5	*Copris minutus*	1.672
6	*Caccobius vulcanus*	3.219
7	*Digitonthophagus bonasus*	3.589
8	*Digitonthophagus gazella*	1.915
9	*Euoniticelles pallipes*	6.131
10	*Euoniticelles fulvus*	4.599
11	*Euoniticelles pallens*	1.743
12	*Gymnopleurus koenigi*	4.687
13	*Gymnopleurus gemmatus*	2.088
14	*Oniticellus cinctus*	3.048
15	*Onthophagus centricornis*	2.309
16	*Onthophagus tragoides*	1.785
17	*Onthophagus cervus*	3.258
18	*Sisyphus crispatus*	3.713
19	*Sisyphus neglectus*	3.964
20	*Tiniocellus spinipes*	15.522

abundant species in the habitat, only 40 % (Table 2) infection was noted among the population. Irrespective of the abundance the infection was pronounced in the dung beetle species. The spore yield recorded highest in *Oniticellus cinctus (3.4x 10^6)* and lowest in *Copris excises (1x10^2).* Of the twenty species of dung beetles showing microsporidian infection, the following eight species were found to be infected heavily infected and suitable host for the microsporidians; *Aphodius conspurcatus, Aphodius prodromus, Euoniticelles pallipes, Euoniticelles fulvus, Euoniticelles pallens , Onthophagus centricornis,* and *Onthophagus cervus.* In these species, the percentage of infection was between 40 - 60%, which is a relatively high percentage in the field condition. These species could be exploited in mass rearing programmes for use of microsporidians in biocontrol applications. The morphological measurements indicate that the mean length and breadth of *Nosema sp.* varied between 4.7 - 2.3 µm x 3.1 0.9 µm respectively and that for *Vairimorpha sp.* between 5.6 4.1 µm x 3.1 2.2 µm respectively.

DISCUSSION

According to Toguebaye and Bouix [6], only ten species belonging to the genus *Nosema* have been described from the family Chrysomelidae. Present identification of the species requires molecular taxonomic tools involving rRNA typing. Microsporidia are important pathogens of insects and are considered to be important regulators of the population dynamics [7] and therefore requires precise identification to augment them in biocontrol programmes. That the microsporidial infection leads to the retarded growth and development [8, 1, 9] reduced food consumption [10], delayed development [11], decreased activity and reduced fecundity [12] in the host insect, make them ideal biocontrol agents. The variation in the size among members of the same genera could indicate that there could be more than one species infecting the host. Conversely, it could also indicate a case of phenotypic plasticity among the species resulting from physiological adjustments to invade the beetle host. Parasitic microsporidia during development utilize energy resources from the host body for their multiplication and development, debilitating the host leading to its retarded growth and development. However, the Microsporidians by infecting the dung beetles in the scrub jungle ecosystem becomes a limiting factor in the population build up of the dung beetles.

CONCLUSION

Dung beetles are ecological important organisms because of their role as decomposers of dung and other decaying organic material and in the enhancement of ecological processes such as dispersal of seeds, nutrient recycling, soil aeration, nutrient transfer as well as breaking down dung and preventing flies from breeding in it.

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