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Identification and morphological characterisation of Silkworm *Bombyx mori* from the region of Amethi, Uttar Pradesh, India

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Abstract
The mulberry silkworm, *Bombyx mori* is the most important silk producing insect in India and exhibits wide diversity in morphological and biometric characters. Besides producing silk, it also has high nutritional value. *Bombyx mori* is a completely domesticated insect and is no longer found in nature. Silkworm belongs to the class Insecta, Phylum Arthropoda because the insect's body is divided into head, thorax and abdomen. For the study on identification and morphological characterization of mulbury silk worm, a total of 85 samples were collected from various sampling sites of District Amethi, Uttar Pradesh, India. These samples were identified with the help of standard taxonomic keys and various morphological characteristics recorded for morphometric analysis. After that these samples were preserved in 70% formalin solution for future reference. The samples collected during various seasons revealed that the morphological characters viz, larval length, width and weight of the silk worm *Bombyx mori* exhibits various growth patterns. The significant variations in the growth patterns were recorded in different seasons, which may be due to the altered seasons and availability of food material mulberry leaves. Results were presented as means ± SD (P values <0.05) were regarded as statistically significant. The present study provides the important information on the morphological variation in various forms of silkmoth *Bombyx mori* in different seasons from the Amethi regions of Uttar Pradesh, India, which is an important parameter in racial investigation of this species of silkmoth.

Keywords: Silkmoth, Bombyx mori, identification, morphometric analysis, Amethi U.P.

1. Introduction
Sericulture is an art of nurturing silkworm for the production of cocoon which is the raw material for the production of silk. Silk is the most cherished of all textiles even today, along with a wide variety of man-made fibre of inimitable excellence as "Queen of Textiles" [1]. Economic interest in this lepidopteran has promoted great progresses in domestication and genetic breeding. The genetic improvement of B. mori is essential in obtaining hybrids with increased productivity and lower defencelessness to various diseases. A silkworm breeding program requires a survey of all the important sericulture characters related to the nature of the egg, larva, cocoon, and moth [3]. The combined strengths of a lepidopteran molecular model and an important economic insect make the silkworm, Bombyx mori, an ideal candidate to initiate intensive genome investigation using potential new experimental strategies now being developed in other systems [6]. The silkworm Bombyx mori is a completely domesticated pest and is no longer found in nature. Currently, several strains of B. mori geographically distributed and genetically improved are available only in germplasm banks distributed about the world, especially where sericulture presents expressive activity. In Brazil, the unique public germplasm bank of B. mori is located at the Universidade Estadual de Maringá, UEM, Paraná, Brazil. The conservation and maintenance of the original characteristics of the species and its different strains in germplasm banks are, therefore, very important, as well as their correct identification [7]. The efficacy of antibiotics against bacterial pathogens of B. mori has been proved already by several authors. Though bacterial infection is well managed by antibiotics, the ability of bacteria to acquire resistance to drugs makes it ineffective within a short duration and hence attempts are being made for the use of plant compounds especially the crude aqueous extracts of plants against silkworm bacterial pathogens. India has a very rich floral diversity yet this potential is not tapped to the fullest extent [8].

2. Review of literature
Jansi rani L, *et al* investigated that the pathogenic microbes isolated from the disease affected larvae, four species of bacteria were isolated and 3 species of fungi also isolated and identified. S. Nageswara Rao, *et al* demonstrated that besides ultrastructural studies, RAPD-PCR can be a useful and reliable tool to detect polymorphism, genetic relationships, and for the identification of the microsporidians. In addition, DNA fingerprints generated in this process have potential applications as diagnostic tools for identification of different microsporidia with considerable accuracy. N.C. Pereira, *et al* studied that the silkworm has accumulated a high level of homozygosity and, consequently, a low level of polymorphisms. This is probably because all cultivated silkworm strains originated from a common ancestor with a long history of inbreeding under consistently strong selective conditions for strain maintenance and improvement. Nevertheless, RAPD analysis has proven to be an efficient technique to detect variability in different B. mori strains. These polymorphisms are useful in the genetic analysis of and discrimination between silkworm strains. Furthermore, RAPD molecular markers can be used to identify strains that are susceptible to the baculovirus BmNPV. G Cermenati, *et al* studied that cultured cells homogenates displayed aminopeptidase N and alkaline phosphatase activity, proving that these two enzymes, involved *in vivo* in the intermediate and final digestion, are expressed also *in vitro*. The columnar cells differentiated in culture were able to internalize two model proteins with quite different transport rates.

3. Materials and Methods

3.1 Biological Material

Twenty B. mori strains of Chinese, Japanese and Indian origins were obtained at the UEM Brazilian germplasm bank, UBGB. In addition, a commercial hybrid of the state of Paraná was included in the analyses, totalling twenty one genotypes (Table 1). Bombyx mori larvae of each strain were reared separately in hygienic and controlled environments, in breeding boxes, with temperature of 28˚C and relative humidity of 50%. Larvae were subjected to artificial lighting with a photoperiod of 14 hours of light and 10 hours of dark. Silkworm larvae were fed with fresh mulberry leaves.

Table 1: *Bombyx mori* strains of the UEM Brazilian germplasm bank, UBGB, according to their geographical origin

Bombyx mori strains			
Commercial hybrid/Brazil	**India**	**China**	**Japan**
Hbd-Bratac	B82	C36	E8
	B106	C75	HA-A
		C211	LM
		C214	KR01
		C121	M8
		C122	M11
			M11-A
			M12-2
			M18
			M18-2
			M19-2

3.2 Herbal Extract Preparation

The powdered herbal products of Eclipta prostrate, Phyllanthus niruri, Punica granatum, acalypha indica and Cannamomum zeylenica were achieved from the medical shop, Amethi. Ten grams of the powder was weighed out and kept in a conical flask soaked with acetone for 6 hrs under air tight condition. The content were then stirred for an hour in magnet stirrer and filtered through a filter paper. The residual extract was collected in a flask and the solvent was allowed to evaporate at room temperature. The extracts were then stored at 4 ^{0}C until use. The resultant residue was then made up to required volume using double distilled water. Similarly, the aqueous extract of the herbal powder was collected using distilled water.

3.3 Scanning electron microscopy

The method employed for scanning electron microscopy (SEM). Purified mature spores (1 x 10^8/ml) of each of the microsporidian isolates were fixed in 2.5% glutaraldehyde prepared in 0.2 Mcacodylate buffer, followed by washing in double distilled water and dehydrated in ethanol series. They were dried in a critical point dryer (EMS-850), coated by gold in sputter coater (EMS-550), mounted on to copper stubs and scanned under JEOL 100· at 15 kV. The scanning electron micrographs pertaining to eight microsporidian isolates studied in the present analysis.

3.4 Mode of transmission

Fifth instar larvae of NB_4D_2 were inoculated per os with purified spores of new microsporidian isolates along with the standard strain. For each of the isolates, two replicates of 50 larvae each were maintained. Each replicate was inoculated with 0.5 ml spore suspension having an infective dose of 1x10^4 spores/ml. The spore suspension was smeared uniformly over the ventral side of fresh mulberry leaves and fed to 50 larvae. The inoculation doses were determined by measuring the ingested portion of the given leaf. The infected larvae were reared till cocooning. The resulting cocoons were collected and kept for moth eclosion. After moth eclosion, mating was allowed for 6 hours and gravid female moths were allowed for oviposition for 24 h. After oviposition, female moths were homogenized individually and examined for Pebrine infection under a phase contrast microscope. The eggs collected from infected moths were treated with cold hydrochloric acid.

3.5 Enzymes assay

Three weeks old midgut cells in culture were pelletted by gentle centrifugation at 400xg for 5 min and re-suspended in a small amount of physiological solution (see above). After three washes, the pellet was re-suspended in a buffer solution (100 mM mannitol, 10 mM Hepes-Tris at pH 7.2) and lysated in the eppendorf vial with a motor for pellet pestle (Sigma). Protein concentration in the lysate was determined according to Bradford (1976) with BSA as standard. All enzymatic assays were conducted under conditions in which products formation depended linearly on enzyme concentration. Aminopeptidase N and alkaline phosphatase activities were determined at 25 °C by measuring the release of p-nitroaniline from L-leucine-pnitroanilide in 40 mM Tris-HCl at pH 7.5 or of pnitrophenol from p-nitrophenylphosphate in 1 M TrisHCl at pH 8, respectively.

3.6 Silkworm strains

The non-diapausing strains are mostly of tropical origin and were characterized by rapid breeding, lower body weight, shorter silk fibre of inferior quality, and resistance to pathogens such as Bombyx nuclear polyhedrosis virus (BmNPV). They undergo 5–6 life cycles/year (polyvoltines). Among the seven non-diapausing strains used in the present study, Pure Mysore (Karnataka state, India), is unique in that it takes more than 28 days to complete its larval life as compared to the 20–22 days observed for almost all of the nondiapausing strains. It spins small, spindle shaped, and light green ish-yellow coloured cocoons. Moria and Sarupat strains (Assam, India), spin small, flossy, and creamish-coloured cocoons of spin dle shape. Daizo, of Chinese origin, makes small, flossy, short, spindle-shaped, dark greenish-yellow cocoons and is characterized by unstable voltinism, i.e., it tends to lay both diapausing and nondiapausing eggs depending upon environmental factors (such as temperature), prevailing during silkworm rearing. Nistari (West Bengal, India) spins small, spindle-shaped, golden-yellow-coloured cocoons.

3.7 Construction of partial genomic library

Genomic DNA from an indigenous silkworm strain, Nistari, was extracted. Silkworm DNA was digested to completion simultaneously with HaeIII, EcoRV, RsaI, and Sau3A1 (Amersham) according to the manufacturer's instructions. The pBluescript SK(+) vector DNA was digested with BamH1 and HindII and ligated to insert DNA and transferred into competent Escherichia coli DH5 α cells using standard procedures. A total of 3513 recombinant clones were picked up by blue–white colour selection from the library. The DNA from the recombinant clones was transferred to nylon membranes by the dot-blot method. Membranes were soaked in 2× SSC for 5 min, then air-dried and used for hybridization.

3.8 Turbidimetry Analysis
Turbidimetry analysis was also carried out for the plant extracts. For this, the standard nutrient broth was prepared and its optical density was read in the spectrophotometer at 550 nm as the initial and 10 ml of the nutrient broth was taken in a clean test tube, inoculated with the bacterial culture isolated from the haemolymph of the diseased silkworm using inoculation loop and a control without bacterial culture was maintained. Similarly, Mueller Hinton broth was taken in different test tubes and was inoculated with bacterial culture. All these test tubes were incubated at 37 C for overnight. They were taken out for the experiment and different volumes of the herbal extract was added into the cultures followed by the addition of distilled water to maintain the volume equal in all test tubes. The test tubes were kept in a shaker for a while and the optical density of the cultures was measured in the spectrophotometer at 550 nm. They were then kept incubated at 37 C and the optical density was measured again at different time intervals. The decrease in the optical density of the culture was taken as an indication of the effectiveness of the herbal extracts against the growth of the bacterial pathogens.

4. Result Analysis
Four colonies isolated and identified using biochemical tests were tabulated in table 1 fungal species also isolated, in a sample of 20 diseased worms about 18 had Aspergillus sps. This indicates that Aspergillosis is a predominant fungal organism affecting Bombyx mori. In order to control the bacterial and fungal infection the sensitivity of isolated microbes from the silkworm was tested against different plant (herbal) extracts of different concentrations.

The evaluation of qualitative and quantitative traits helps characterize and differentiate

Between different strains from the UEM Brazilian Germplasm Bank. In addition to helping to

Maintain the germplasm bank, these analyses are crucial in choosing the parents involved in the production of superior hybrids in breeding programs. Moreover, the biological performance of B. mori reflects maximum final silk production potential. Qualitative traits from 14 silkworm strains were evaluated, including ES, CNL, presence of LM, CS, CC, PC, PS, female moth color, and male moth color; the results are listed in Table 2. ES varied according to the geographical origin of the strain; Japanese strains had elliptical-shaped eggs, whereas Chinese strains had oval-shaped eggs. Most of the eggs were small in size, except for AS3 and C75 strains. Many of the CNL were brown in color. Exceptions included the AS31, C36, C37, and JK strains, whose CNL were black. Two patterns of LM, plain and multilunar, were observed. Plain larvae did not have any markings, whereas multilunar larvae had 2 different characteristic spots. In the literature, different types of LM have been described, including zebra, speckled, quail, multistars, and others. LM is a useful variable as it helps prevent the mixing or contamination of larvae from different strains.

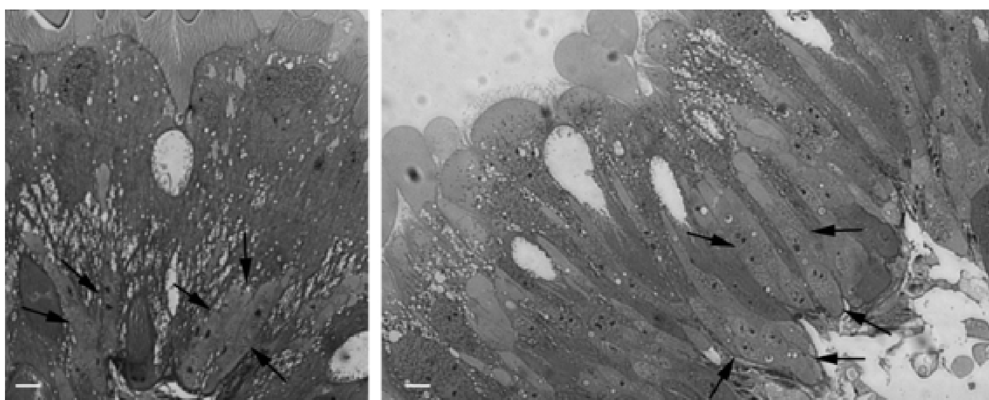

Fig 1: Semithin sections of the midgut epithelium of Bombyx mori

5. Conclusion
All the twenty microsporidian isolates differed in their pathogenicity, dimensions of the spores, number of rows and coils of polar filament and their organisation, as well as the RAPD profiles indicating strain variation among different microsporidian isolates. Further, RAPD-PCR is found to be delicate and worthwhile tool to distinguish between different microsporidia and a valuable addition to existing methods like RFLP and SSU-rRNA sequence analysis in resolving genetic variation among different microsporidians infecting commercial silkworms.

The present study is a step towards harnessing highly informative microsatellite loci that provide a ubiquitous marker system for characterization of silkworm genome. An additional usage of microsatellite markers in silkworm is their potential application as markers for the related silk-secreting wild silk moths, which belong to the family Saturniidae. We have evidence to show that a majority of the Bombyx micro satellite primers effectively amplify the DNA of the wild silk moth species. Such a study would facilitate the genetic analysis of the wild silk moths, some of which are endangered and the genetics for which is little known.

6. References

1. Jansi rani L. Impact of certain herbal extracts against bacterial disease of silkworm Bombyx mori L, International Journal of Research in Pharmacy and Pharmaceutical Sciences. 2016; 1(1):08-10.
2. Nageswara Rao S. Genetic diversity and phylogenetic relationships among microsporidia infecting the silkworm, Bombyx mori, using random amplification of polymorphic DNA: Morphological and ultrastructural characterization, Journal of Invertebrate Pathology. 2007, 193-204.
3. Pereira NC. Biological and molecular characterization of silkworm strains from the Brazilian germplasm bank of Bombyx mori, Genetics and Molecular Research, 2013,

2138-2147.
4. Awasti AK. Molecular evalution of bivoltine, polyvoltine and mutant silkworm (Bombyx mori L.) With RAPD, ISSR and RFLP-STS markers, Indian journal of Biotechnology. 2008; 7:188-194.
5. Cermenati G. A morphological and functional characterization of Bombyx mori larval midgut cells in culture, Research Report, 2007, 119-126.
6. Damodar Reddy K. Microsatellites in the silkworm, Bombyx mori: Abundance, polymorphism, and strain characterization, Genome, 1999; 42:1057-1065.
7. Verônica Aureliana Fassina. Low Genetic Polymorphism at the Cytochrome C Oxidase I in Silkworm Strains of the Brazilian Germplasm Bank, Open Journal of Genetics. 2014, 202-209.
8. Karthikairaj K. Efficacy of some herbal extracts on microbes causing flacherie disease in mulberry silkworm, Bombyx mori L., 2014, 89-93.