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Overview on neo classical aspects of Indian uzifly, *E. sorbillans*: The notorious pest of silkworm *Bombyx mori* L.

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Sericulture is a major vital and viable agro-based cottage industry across Asia. uzifly, *Exorista sorbillans* (Wied.) is one of the polyphagous endoparasitoid of the silk-producing lepidopteran *Bombyx mori* L., which causes 20-30% cocoon crop loss across India and other silk producing Asian countries. Earlier studies confined only towards understating its biology and developing control measure to check its infestation of this pest. Still there is large gap that remains to be filled mainly with reference to the classical, cytological and molecular aspects of the fly pest. The article anatomizes two decade effort the various research findings of our group. The focus of the article is on mainly neo classical aspects of *E. sorbillans* and its interactions with symbionts *Wolbachia* and *Wo* phage along with scope for future research.

Sericulture is a major vital and viable agro-based cottage industry across Asia. It is one of those key industries which provide gainful employment to weaker sections of the society and earns high foreign exchange in many developing countries of the world. However in India, this industry looses 20-30% of its crop due to a notorious pest *E. sorbillans* which parasitizes on silkworm *Bombyx mori* L. The silkworm larvae parasitized by uzifly, dies either before or after spinning the cocoons based on the larval instar of the silkworm. Around 350-400 macroscopic eggs are laid by a single female on the lateral sides and in between the integuments of the larvae. Upon hatching these uzi maggots pierce into silkworm leaving black scars on the larval exoskeleton. If the infestation is during the fifth instar, the worms manage to spin the cocoon but fail to metamorphosis into pupae. The developing uzi maggots inside bore out of the cocoons rendering them unfit for reeling.

With uzifly being such a notorious pest, great attention has been given in understating the biology of this pest and in developing some control measure to check its infestation. However, all these studies do not address various aspects of the tachinid parasite. Still there is large gap that remains to be filled mainly with reference to the classical, cytological and molecular aspects of uzifly *E. sorbillans*. The article anatomizes the various research findings of our group. The focus of the article is on classical and applied aspects of *E. sorbillans* like Bionomics, Cytotaxonomy, Molecular divergence, Control measures and Host microbe interactions. The article is important for all interested researchers as it provides holistic knowledge about various facets of uzifly biology.

A detailed account of morphology on all the holometabolus stages of uzifly was studied by Manjunath and Pruttaraju (1993). It made significant contribution in describing in depth structure of uzi egg, along with the remaining stages (maggot, Pupae and Adults). The finding was first of its kind in uzifly fly and emphasized the importance of hatching pleat during the emergence of the embryos. Further, Puttaraju and Chowdahi (1984) investigated the the diploid chromosome no 2n=12. The Karyotypic analysis first time revealed the 5 pairs of submetacenrtic autosome and pair of allosome *i.e.* acrocentric X and submetacentric Y. This finding served as "Cytological identity card" for uzifly. Later various tissues were isolated and cytological studies were carried out at different stages of *E. sorbillans* for mitotic, meiotic and polytene chromosome. The behaviour of chromosomes during gamatogenesis gave new insights into the developmental biology of the fly (Puttaraju and Majunath 2003). Further comprehensive chromosomal banding by C and G banding technique (Venkatachalapath and Puttaraju, 2000) across the south Indian uzifly population revealed significant cytological difference which led the investigators to hypothesize the possible existence of cryptic species within *E. sorbillans.*

Cryptic genetic variation is the dark matter of biology, normally less probed, but might be an essential source of physiological and evolutionary potential. Earlier studies suggest that identification of cryptic species if any in the

uzifly may be helpful for investigating better strategies to combat the uzifly menace on silkworm.Apart from that, it has significance on applied aspects of biology like, biodiversity assessment and conservation, parasite/host relationships, antidotes for poisons, eco-toxicology tests etc.

With the advent of novel molecular techniques like RAPD the significant heterogeneity in the south Indian uzi pest populations was identified (Prakash and Puttaraju, 2009). This led to the advent of molecular signatures in uzifly and emphasized microevolution in uzifly. All the above finding epitomized the various stages for devising control measures to check the proliferation of this pest. The physical method of use of Gama radiations and the chemical method Diflubenzuron (chitin inhibitor) for management of uzifly were found to be appropriate method for controlling this pest at field level. However there were few limitations in both the methods and there was a craving need for a better and improved biological method.

Our group in 2001 explored the association *Wolbachia*, a ubiquitous reproductive manipulator for the first time (Madhu and Puttaraju, 2001). The uzifly-*Wolbachia* interaction revealed high level of intimacy and exhibited mutualistic association (Prakash and Puttaraju, 2009). *Wolbachia*-uzifly are interdependent for their survival and reproduction, respectively. *Wolbachia* provides both positive and negative fitness benefits to uzifly like fecundity enhancement, Cytoplasmic incompatibility, Nucleocytoplasmic conflicts, sex ratio distortions and so on. *Wolbachia* induced cytoplasmic incompatibility in uzifly. When *Wolbachia* is eliminated it resulted in reduced reproductive fitness in the hosts. This heavy dependence of uzifly on *Wolbachia* for its reproduction paved way for a novel management technique. When *Wolbachia* was targeted by antibiotic treatment (tetracycline) through Silkworm diet (Mulberry leaves) it not only curtailed uzifly but also improved the qualitative and quantitative characteristics of silkworm cocoon production (Prakash and Puttaraju, 2006). Further the diversity of *Wolbachia* infections in uzifly population was assayed across India. Novel strains were identified and it was interesting to know that all the uzifly populations were doubly infected with *Wolbachia* A and B super group. The bio-assay crossing studies suggested that the endosymbiotic reproductive manipulator *Wolbachia* induces variety of reproductive anamolies in Uzifly (unidirectional CI, fecundity enhancement) which are regarded as a raw material for microbial mediated speciation. Our investigation sheds light on the possible role of *Wolbachia* in diversification of uzifly.

Fig. 1 : A) uzifly infestation silkworm, B) uzi black scars on silkworm body C_1-C_2)Male and female uzifly Karyotype. C_3-C_4) C and G banding pattern, D) *Wolbachia* endosymbiont in uzifly

Interestingly *Wolbachia* of uzifly in turn infected with bacteriophage WO (Guruprasad *et al.*, 2011). The sequence analysis of the phage outer capsid protein gene ORF-7 shows significant diversity in uzi population collected from different parts of the India (Unpublished data). These findings crucial and in control of uzifly and is vital in understanding its evolution.

Based on the current understandings several new avenues have opened up for future research and several new dimensions that are to be explored. Given below are few of the research areas that are to be investigated in detail.

– Transinfection of novel *Wolbachia* strains into *E. sorbillans* or from *E. sorbillans* to other hosts could provide interesting insights on dynamics of *Wolbachia* under different host background.

– Uzifly is polyphagous insect and successfully completes its lifecycles on 10 different lepidopteran insects/ pests other then silkworm *Bombyx mori*. Therefore to study Host-associated differentiation (HAD) cascade in Uzifly, the parasitoid have to be reared on different hosts for many filial generations and then these populations have to be characterized through molecular and bio-assay experiments

– Further wide-ranging survey in tracing *Wolbachia* single infection and carrying out the Multi lo*cus sequence* typing among the different populations of Uzifly, *E. sorbillans* will be helpful in Knowing clear genetic architecture and better understanding of *Wolbachia*-Uzifly interaction.

– The influence of *Wolbachia* in Uzifly could also be investigated on non-reproductive fitness traits such as

survival or protection against natural enemies.

– Studying the co-evolutinory pattern in tripartite association between the host-parasitoid-endosymbiont.

– Identification of the Wolbachia and Phage *WO* diversities.

Conclusion : The article overviews on classical, cytological and molecular aspects of tachinid uzifly it is important for all researchers and interested people. It provides holistic knowledge about various facets of uzifly biology.

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