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Overview of distribution and diversity of *Wolbachia* endosymbiont among the different geographical populations of Indian Uzifly, *Exorista sorbillans* (Diptera: Tachinidae)

H.C. Huchesh and H.P. Puttaraju

ABSTRACT
Microorganisms are unique life forms having great impact on functioning and perpetuation of biosphere. Among them, *Wolbachia* is ubiquitous gram negative rickettsial endosymbiont taking its shelter in somatic and reproductive tissues of class insecta. *Wolbachia* induces variety of phenotypes within insects from reproductive parasitism to mutualism. Due to the infection dynamics of *Wolbachia* and its ability to induce reproductive anomalies in their hosts, it has been touted as the most effective bio-control agent for management of insect pests and vectors. However, for implementation of any *Wolbachia* based bio-control strategies it is essential to know its distribution, diversity and interactions with their hosts. *Wolbachia* interactions in Uzi fly *Exorista sorbillans* are well documented and are proposed as agents for management of this pest. However, their distribution and diversity across the entire Indian sub-continent is left void. In the current study 31 populations of Uzi fly collected from the Mulberry Silkworm belts of India have been screened and characterized. 213 samples were screened and infection dynamics in the individuals have been documented. The findings suggest high prevalence of AB *Wolbachia* super infections in field populations of Uzi fly. This important update on the infection status and diversity of *Wolbachia* is of greater significance for implementation of the previously proposed *Wolbachia* based bio-control strategies of Uzi fly in India.

Keywords: *Wolbachia, Exorista sorbillans,* Bio-control, Positive fitness, *Wolbachia* surface protein.

1. Introduction
Microorganisms are unique forms of life, omnipresent in all environments. Among the most prevalent microbes, bacterial endosymbionts are very special in insects, as they inflict various types of metabolic, physiological and reproductive alterations in their host [1]. Their role in pesticide degradation, vitamin synthesis, pheromone production and pathogen prevention has been documented [2, 3]. *Wolbachia* is one such ubiquitous gram negative, maternally transmitted rickettsial endosymboint, that exhibit obligate intracellular mode of life style infecting up to 76% of all known insects [4, 6]. *Wolbachia* are known to induce diverse range of phenotypes in infected host ranging from reproductive parasitism to mutualism. The familiar reproductive phenotypes by *Wolbachia,* includes, Cytoplasmic Incompatibility, Parthenogenesis Induction, Male Killing, Feminization [5]. Additionally, *Wolbachia* are also known to provide certain fitness benefits to their hosts like, higher rates of fecundity, protection against various viral pathogens and programmed cell death in ovary of some insects [7, 8, 9]. At present, *Wolbachia* have been divided into twelve supergroups primarily based on phylogenetic data from the conserved WSP genes sequence. Among them A and B Super group *Wolbachia* found in insects. Some insects naturally harbor a single infection, A or B type or double infection with a representative from each subdivision (A and B). These interesting associations of *Wolbachia* with their hosts have opened new promising avenues for its exploitation in both basic and applied research. However the molecular mechanism underlining the diverse phenotype is poorly known and these phenotypes are transient across the varied hosts and appear to be influenced by both host and *Wolbachia* intrinsic factor.
Wolbachia have been touted as the most efficient bio-control agent as it can be effectively employed in population suppression and replacement strategies. With the advent of precise diagnostic and molecular tools the scope for better exploitation of *Wolbachia* has increased.

Recently, the potential application of the *w*Melpop (*Wolbachia* strains that shorten insect longevity) to control *Aedes aegypti* mosquitoes has been proved successful [10]. Additionally, recent literature suggests that *Wolbachia* inhibits viral replication in *Drosophila* [11] and mosquito vectors [12]. Earlier studies by various researchers on uzifly have suggested that *Wolbachia* can be effectively used in control of Uzi fly, *Exorista sorbillans* [13, 14].

The Uzi fly, *Exorista sorbillans*, a notorious pest of Silkworm causing an estimated crop loss of 30%. Uzi fly is distributed across 22 different countries and has significant impact on sericulture industry [15], thus gaining much attention from the scientific community to control this notorious pest. The Uzi fly can be seen distributed across the varied geographical mulberry growing sectors of India (Fig.1) experiencing different agro-climatic conditions. Several strategies have been employed for controlling Uzi fly populations, including physical, chemical and environmental management methods. However, none of these methods are effective and are crippled with few limitations. There is craving need for a better and improved biological method to control Uzi fly. Earlier investigators have screened the populations of Uzi fly and evaluated its Cytoplasmic Incompatibility. It has been proposed that *Wolbachia* can be effectively used for Bio-control of Uzi fly *Exorista sorbillans*.

Earlier literature on *Wolbachia* infection dynamics in several insect populations suggests that there is incongruence in the distribution and diversity of *Wolbachia* [16, 17] restricting the implementation of bio-control strategies. The distribution and diversity of *Wolbachia* infections in Uzi fly populations across all the populations is unavailable. Therefore, in the current study, 31 populations of Uzi fly have been collected representing the major Mulberry Silkworm belts of India and screened and characterized. This important update will be influential in implementation of *Wolbachia* based bio-control strategies in India.

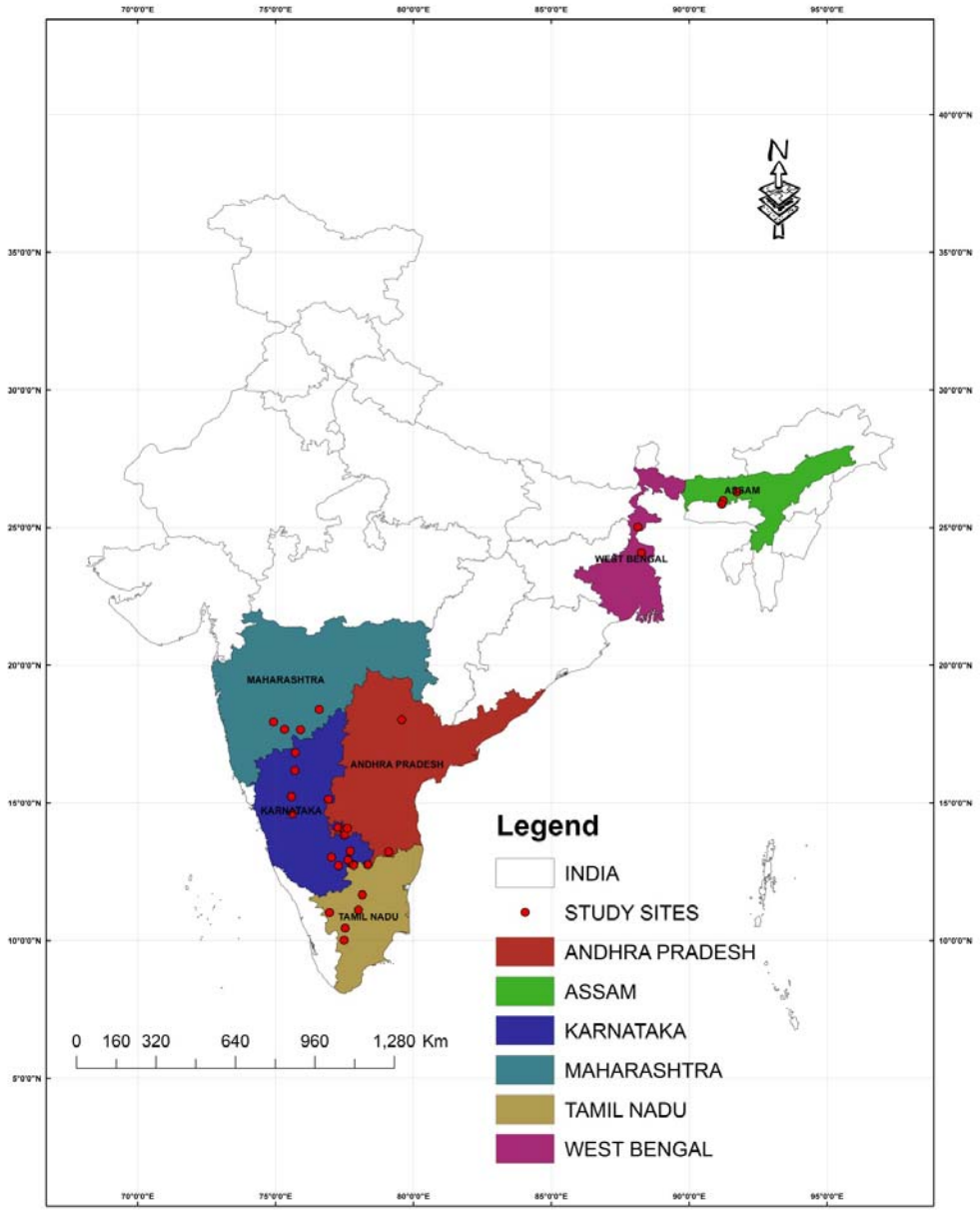

Fig 1: The populations collected from different geographical study areas are represented in dots

2. Materials and methods

2.1 Collection of Uzi fly from the entire Mulberry Silkworm belts of India.

The Uzi maggots were collected from 31 locations across Southern, Central, Northern, North Eastern zones of India from various silk markets/ villages/research institutes located in six different states of India Viz., Karnataka, Andhra Pradesh, Tamil Nadu, Maharashtra, West Bengal and Assam ranging between the geographical region $11^0.16^1$-$25^0.39^1$N & $74^0.57^1$-$91^0.10^1$E (Table 1). The maggots were allowed to pupate in the aerated plastic containers partially filled with sand. The puparium were transferred to insect rearing cages after the third day of pupation. The emerged flies were fed through cotton soaked in 8% sucrose. The adults were maintained in standard insectaria conditions of 25 ^{0}C temperature and 65%-80% RH.

Table 1: Collection of Uzifly samples across different geographical locations of India

State & Zones	Location	Latitude	Longitude	Sample size (n)	Only A	Only B	%AB
Karnataka (South)	Attibale	$12^0.42'$N	$77^0.07'$E	10	0	0	10(100%)
	Bagalkot	$16^0.18'$N	$75^0.69'$E	12	0	0	12(100%)
	Bellary	15.15′N	$76^0.93'$E	06	0	0	06(100%)
	Bijapura	16°.83′N	$75^0.71'$E	09	0	0	09(100%)
	Pavagada	$22^0.46'$N	$76^0.40'$E	10	0	0	10(100%)
	Devanhalli	13.23′N	$77^0.7'$E	07	0	0	07(100%)
	Kunigal	$13^0.01'$N	$77^0.46'$E	10	0	0	10(100%)
	Ramanagara	$12^0.43'$N	$77^0.o8'$E	11	0	0	11(100%)
	Shirahatti	$15^0.23'$ N	$75^0.58'$ E	09	0	0	09(100%)
	Ranibenuur	$14^0.33'$ N	$75^0.33'$E	08	0	0	08(100%)
	Taagchiguppae	$12^0.91'$ N	$77^0.47$ 'E	10	0	0	10(100%)
Tamil Nadu (South)	Housur	$12^0.43'$N	$77^0.49'$E	06	0	0	06(100%)
	Palini	$10^0.45'$N	$77^0.51'$E	06	0	0	06(100%)
	Sellum	$11^0.65'$N	$78^0.16'$E	04	0	0	04(100%)
	Theni	10°04′N	77°45′E.	08	0	0	08(100%)
	Velour	$12^0.55'$N	$79^0.08'$E	05	0	0	05(100%)
	Coimbatore	$11^0.16'$ N	$74^0.57'$E	08	0	0	08(100%)
Andhra Pradesh (South)	Chittor	13°12′N	79°07′E	07	0	0	07(100%)
	Hindupura	$13^0.50'$N	$77^0.49'$E	04	0	0	04(100%)
	Kuppam	$12^0.75'$N	$78^0.37'$E	05	0	0	05(100%)
	Penukonda	14.08′N	$77^0.58'$E	07	0	0	07(100%)
	Pulmnera	$13^0.02'$N	$78^0.02'$E	08	0	0	08(100%)
Maharashtra (Central)	Latur	$18^0.24'$N	$76^0.33'$E	08	0	0	08(100%)
	Pandrapura	$17^0.40'$N	$74^0.57'$E	07	0	0	07(100%)
	Solopur	$17^0.68'$N	$75^0.92'$E	05	0	0	05(100%)
	Malsirus	$17^0.92'$N	$74^0.55'$E	03	0	0	03(100%)
West Bengal (North)	Malda	$25^0.39'$N	$89^0.49$'E	03	0	0	03(100%)
	Berhampore	$24^0.02'$N	$88^0.36'$E	06	0	0	06(100%)
Assam North-East	Azhra	$26^0.12'$N	$91^0.06'$E	03	0	0	03(100%)
	Boko	$25^0.95'$N	$91^0.25'$E	08	0	0	08(100%)
	Hahim	$20^0.15'$N	$91^0.10'$E	02	0	0	02(100%)
Total				**213(100%)**			**213(100%)**

2.2 DNA isolation

The flies were surface sterilized by three sequential washes in 30% H_2O_2, 95% ethanol and sterile water and were subjected to DNA extraction using Zymos DNA extraction kit. DNA was isolated from thorax of Uzi fly as per the manufacturers protocol. The isolated DNA was quantified through agarose gel electrophoresis (0.8%) and spectrophotometry. The quantified DNA was further used for diagnostic amplification of *Wolbachia* through PCR.

2.3 Amplification of *Wolbachia* surface protein (WSP) genes

The DNA extracted from different Uzi fly populations was subjected for diagnostic PCR assay by amplifying the WSP gene (*Wolbachia* surface protein gene) with specific primers [18]. PCR assay was carried out in an Eppendorf thermocycler with 25 µl of reaction volume containing 2.5 µL of 10X PCR buffer, 0.4 mM of each dNTP, 4 mM of $MgCl_2$, 1 µl of forward and reverse primers (20 pmol) each, 0.5 IU of Taq DNA polymerase (New England Biolabs, England) and 20 ng of template DNA. The primers WSP 81F 5'TGG TCC AAT AAG TGA TGA AGA AAC and wsp 691R 5' AAA AAT TAA ACG CTA CTC CA for general (600bp) and WSP136F 5'-TGA AAT TTT ACC TCT TTT C-3' and 691R 5'-AAA AAT TAA ACG CTA CTC CA-3' for *Wolbachia* A supergroup (550 base pairs) and 81F 5'-TGG TCC AAT AAG TGA TGA AGA AAC-3' and 522 R 5'-ACC AGC TTT TGC TTG ATA-3' for *Wolbachia* B supergroup (450 base pairs) were used. The PCR profile employed is as follows: denaturation at 95 °C for

3 min, followed by 40 cycles of 95 °C for 30 sec, 50 °C for 30 sec, and 72 °C for 30 sec and the final extension at 72 °C for 7 min. Double-distilled water instead of DNA samples was used as negative control.

3. Results and Discussion

A total of 213 Uzi fly samples were screened for *Wolbachia* infections from 31 populations across various geographical regions of India. Diagnostic PCR was performed through general WSP primers. All the Uzi fly populations collected from wide geographic areas revealed 100% *Wolbachia* infections. Further characterization through A and B supergroup specific primers revealed that all individuals were homogenously superinfected with AB super group (Table: 1 and Fig: 2-4). The purposive sampling survey included 31 locations that covered the major mulberry sericulture belts of India, between 10°04'N-26°12'N and 75°33'E-91°25'E geographical indications with distinct agro climatic conditions like type of soil, seasonal variation, temperature, humidity, rain fall, longitude and latitude. This is the first report assayed across India on distribution and diversity of *Wolbachia* infections in Indian Uzi fly [21].

Wolbachia provides both positive and negative fitness benefits to uzifly like fecundity enhancement, Cytoplasmic incompatibility and *Wolbachia* causes number of reproductive alterations with its host and thereby play a crucial role in ecology, evolution and reproductive biology of its host [20]. When *Wolbachia* infections are cured through Silkworm diet with antibiotic feeding (Mulberry leaves) it not only curtailed Uzi fly but also improved the qualitative and quantitative characteristics of silkworm cocoon production [21]. This heavy dependence of Uzi fly on *Wolbachia* for its reproduction paved way for a novel management technique.

All the individuals of screened Uzi fly were doubly infected with A and B super groups of *Wolbachia*. Earlier it was hypothesized that whenever, multiple strains of *Wolbachia* co-exist in a single host, there must be intra endosymbiotic conflicts leading to elimination/ death/ excessive dominance of one of the super groups. However, studies suggest that interactions among the symbionts themselves probably play a critical role in determining the distributions of symbionts in natural populations [22]. Multiple infection in females enables them to have a longer life span, have higher egg hatch in compatible crosses, and are more fecund relative to singly infected females [23]. The reproductive advantage afforded to infected females can permit the *Wolbachia* infection to spread, replacing the cytotype of the host population [24]. Thus the possibility of double infections in all the Uzi fly populations is justified. However, it would be interesting to see in future if there are any possibilities of single infections in Uzi flies.

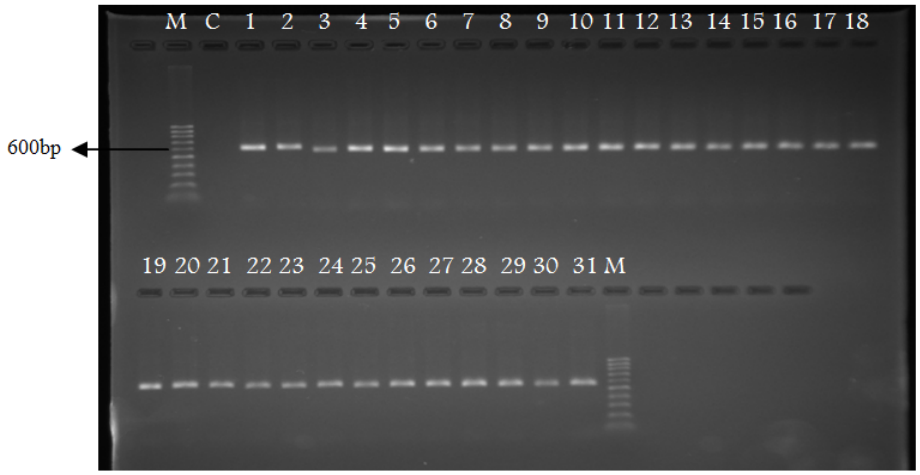

Fig 2: *Wolbachia* infection in different populations of Uzifly across India by using general WSP primer.

Fig 3: *Wolbachia* A super group in various populations of Uzifly collected from different geographical locations of India

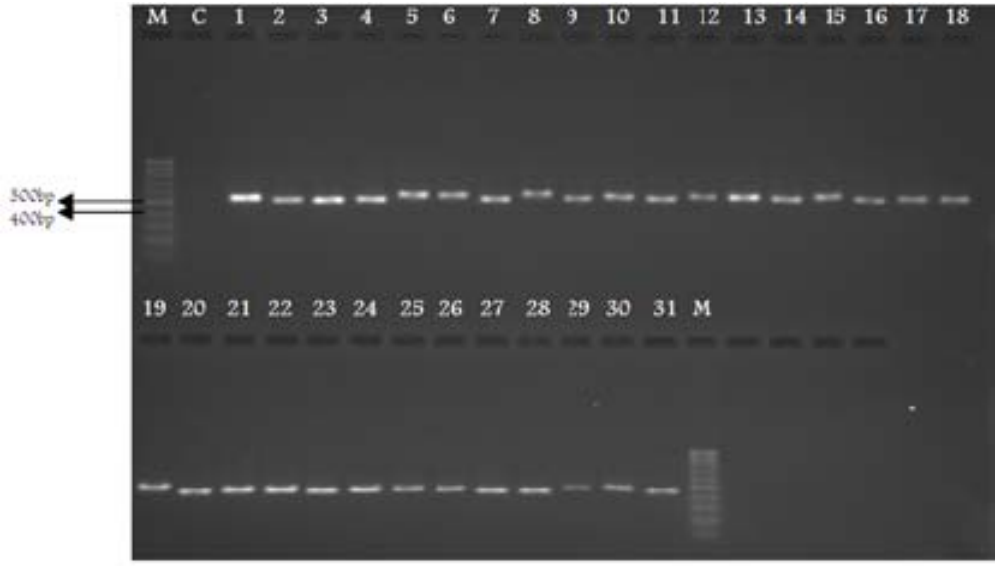

Fig 4: *Wolbachia* B super group in various populations of Uzifly collected from different geographical locations of India.

However, implementation of any *Wolbachia* based control strategies completely depends on the distribution and diversity of *Wolbachia* infection dynamics in field population. The distribution of *Wolbachia* is not restricted to few insect orders but is found in almost all the insect orders and this is a key aspect for its evolutionary success. *Wolbachia* infections are always transient and vary over evolutionary time scales. Infections in insect populations keep on fluctuating due to either higher exposure to natural antibiotics, temperatures or intrinsic properties of different insect orders. Additionally, upon entering the novel hosts *Wolbachia* are known to switch their roles so that they benefit themselves and the insect hosts [25, 26]. In the current study all the Uzi fly populations have been screened for *Wolbachia* infections and characterized to understand the infection dynamics to implement bio-control strategies. The update would be important in devising population suppression strategies by reducing the threshold infection frequency required for *Wolbachia* invasion and increasing cytoplasmic drive rates.

4. Conclusion

A total of screened 31 populations of Uzi fly were screened for Wolbachia infection, *Exorista sorbillans,* which infests the silkworm *Bombyx mori.* All screened population are doubly infected with AB super groups *Wolbachia*. *Wolbachia* provides some positive and negative effects in Uzi fly. Such as enhancing fecundity in infected Uzi flies, while cause cytoplasmic incompatibility in cured females irrespective of the presence of *Wolbachia* in males. they play a crucial role in ecology, evolution and reproductive biology of its host. *Wolbachia* targeted tetracycline treatment to silkworm enhances silkworm fitness, while decrease reproductive fitness of the Uzi flies. Therefore current study signifies substantiate the presence of *Wolbachia* throughout Indian subcontinent for devising effective management strategies of the Uzi fly, *Exorista sorbillans.*

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