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Dr. C.A. Viraktamath

Featuring

- Journey of two eminent taxonomists
- Bacterial toxins for pest management
- Student corner

Dr. Dhriti Banerjee

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Editorial Board

***Cover page image: Dr. Santhoshkumar C Kedar (CSIR - Central Institute of Medicinal and Aromatic Plants), Lucknow -226015, Uttar Pradesh, showing emergence of Cicada (*Chremistica* sp.).**

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CONTENTS

JULY 2022 | VOL 3 | ISSUE 2

EDITORIAL

Communicating entomology to common public: Way forward

The Indian Entomologist, an initiative by the young minds under the auspices of the Entomological Society of India has so far brought out five issues and this is the sixth. I see dramatic improvements in the logistics and deliberations of these issues. In fact it has multiple and varied contents to make it unique. That is why I wrote in the editorial note of the last issue that the Indian Entomologist is serving as a unique forum for dissemination of Entomology and it is progressing well in this direction. I also stressed about the introspection of what we all are doing as professional Entomologists. I wish to continue stressing about this introspection, especially in assessing how we are moving forward in comparison to what is going on in other such platforms. Let me do this by taking just one example, the "Entomology Today" brought out by the Entomological Society of America and "Indian Entomologist" by ESI. Needless to mention this will hover around "effective science communication".

"Entomology Today" brings out interesting discoveries in the world of insect science, news and events from entomological societies, in addition to feature articles by volunteer authors. In this manner it is connecting Entomologists and others, who study insects, their importance, and their impact. One can see effective science communication rampant herein. In similar lines Indian Entomologist had also brought out blogs to bring day to day updates. This is to enable people connected with recent updates in Indian Entomology. Our blog section is one of the unique platform for the Entomologists to bring to the fore their communication skills. This is also serving the common public with updated happenings in Entomology. Till date, Indian Entomologist has published more than 38 blogs which cover different topics contributed by many authors including students. This section attracted the unique viewer base for the Indian Entomologist across the world. However, there is need to attract more students and young minds to write blogs.

Thus, it is an opportunity for Entomologists "to communicate". The authors need to care about answering questions on- Why should the reader care about our writing? Whether the writing by us, the professional Entomologist, is informal? Is it brief, to the point and sticks to the basics of what readers need to know? As reiterated in the Entomology Today and Indian Entomologists blogs', sharing Entomology as a science here cannot be purely academic, as the readers do want to peek into the science behind. Our writing must provoke the reader to "know" about our research, especially those which arise as surprising facts, and those warranting further research. By default, these must include visuals that are effective in communicating and in as little space as possible. What and how we communicate must be based on the target audience and the goal to be achieved. Successful science communication can be less straightforward and simpler than we presume. In the contents of Indian Entomologist it is high time that we introspect on all these points. The objective is to communicate Entomology to the common people effectively. In addition, as I had been stressing always, these shall not only perceive how Entomology was performed and disseminated by our earlier generation of Entomologists, but also about what imminent changes are warranted to ensure effective communication. Let us forge ahead with this in mind in our future issues of the Indian Entomologist.

V V Ramamurthy
Editor in Chief, Indian Entomologist

Photorhabdus bacterial toxins as a candidate for insect pest bio-management: an update

Tushar K Dutta, Victor Phani and Abhishek Mandal

Abstract: Insect-parasitic nematodes belonging to the families Heterorhabditidae and Steinernematidae have developed symbiotic relationships with bacteria belonging to the genera *Photorhabdus* and *Xenorhabdus*, respectively. The nematode–bacteria complex can kill the host insect within 24-48 h via septicemia or toxaemia. Bacteria (which lives in the nematode gut) enter the host through its nematode partner, and the nematode relies on bacteria for nutrition from liquefied host tissue. The bacteria kill the insect by using its arsenal of toxins and secondary metabolites. These nematodes have long been utilized as insect biological control agents, but their short shelf life and demand for a certain temperature and moisture range for field performance have limited their commercial use. A number of protein toxins from distinct Indian strains of *Photorhabdus* spp. were identified in our laboratory. The biological activity of candidate toxins was tested in *Galleria mellonella*, a model insect, and then in agriculturally important insects such as *Heliocoverpa armigera*, *Spodoptera litura*, *S. exigua* and *S. frugiperda*. When toxins was administered orally either via artificial diet or force-feeding, the toxins had shown catalytic activity on the insect gut epithelial cells and moved to hemocoel by proteolytically cleaving the basement membrane lining in the gut-hemocoel barrier. Following that, a cytotoxic effect on immunocytes or hemocytes was seen, similar to apoptosis or cell death. Toxins also had an immunomodulatory effect as documented by the elevated phenoloxidase activity in the hemolymph. These gut-active toxins putatively interact with different insect midgut receptors. Currently, we are pursuing RNAi knockdown of receptors to establish their role in disease development. The novel insecticidal toxins characterized from *Photorhabdus* spp. may provide a greater diversity of biotoxins at disposal for pest management either via transgenic mean or bio-protectant formulations.

Key words: Txp40; TcaB; insecticidal toxin; entomopathogenic nematode; insect gut receptor

The explosion of world's human population (an exponential increase from initial estimate of 0.35 billion during 14th century to current 7.9 billion that may reach up to 9 billion by 2050) has put an unrealistic demand on global crop production especially because of the shrinking arable land and a push towards water-intensive agriculture due to water scarcity. In parallel, insect pests consume approximately 20% of global agricultural yield. This problem is aggravated due to certain factors such as global climate change, cultivation of high input-requiring plant varieties and indiscriminate usage of chemical insecticides (Shankhu *et al*., 2020; Dutta *et al*., 2021a). An increased regulation of synthetic pesticides towards deregistration and restricted use is occurring frequently because of some alarming issues like pesticide resistance development in insects, adverse effect of insecticides on the environment and soil health, greater half-life of common insecticides in soil and non-specific selectivity of insecticides in target organism with only handful of molecular targets including acetylcholinesterase (AchE) enzyme, voltage-gated sodium (Na_v) ion channels and glutamate-gated chlorine channel or Gamma-Aminobutyric Acid (GABA). Consequently, current growers are facing shortage of active insecticides amid the increasing incidence of newer insect pests.

For the control of insect pests in agriculture, alternative tactics such as the production of transgenic crops producing insecticidal protein have been used to great success. Today, the most successful protein toxins used in the development of transgenic crops are those produced by the bacterium *Bacillus thuringiensis*, which produces an insecticidal crystalline protein known as Bt delta endotoxin (also known as Cry toxin) that kills insects (Bravo *et al*., 2011, 2015). As a result of the widespread use of Bt-based biopesticides on a broad scale, as well as the commercial cultivation of transgenic plants expressing toxin genes, resistant insect populations are being emerged in countries such as India, China, and the United States (Tabashnik, 2015; Tabashnik and Carrière, 2017; Carrière *et al*., 2019). Thus, development of novel protein toxins is necessary in order to increase the diversity of genes available for pest control applications.

Biocontrol agents against insect pests, such as Entomopathogenic nematodes (EPNs), have been utilized for quite long, and the bacteria that they carry have been investigated in greater depth (Clarke, 2020). There are two bacterial genera, i.e. *Xenorhabdus* and *Photorhabdus*, which live in close association with nematode genera, *Steinernema* and *Heterorhabditis*, respectively, as symbiotic partners. Numerous insects, such as members of Coleoptera, Lepidoptera, Diptera, and Dictyoptera order can be infected and killed by this nematode-bacterium duo (Lacey *et al*., 2015; Garcia-del-Pino *et al*., 2018).

The symbiotic bacteria are harboured in the intestine of nematode infective juveniles (IJs), which are free-living stages. IJs scavenge for insect hosts in the soil and enter the hemocoel of the host through natural orifices or by penetrating the cuticle. The worms then liberate their symbiotic bacteria that proliferate rapidly and eventually kill the host insect within 24 to 48 hours. To complete development, worms feed on bacteria and dead insect tissues. After 2-3 generations, when the insect cadaver is deprived of nutrients and nematode densities approach critical levels, IJs harbouring their symbiotic bacteria emerge from the cadaver in quest of fresh host insects. These bacteria are quite fascinating in the bacterial world because they form mutualistic associations with one host, nematodes, and a harmful association with another phylum, insects (Clarke, 2020). A schematic representation of life cycle of an EPN (*Heterorhabditis indica* that harbors the bacterium *Photorhabdus akhurstii*) in a model insect, *Galleria mellonella* is given in Fig. 1.

The motile gram-negative bacteria *Xenorhabdus* spp. and *Photorhabdus* spp. are member of Morganellaceae family that belong to Gammaproteobacteria class (Machado *et al*., 2018). *Xenorhabdus* spp.and *Photorhabdus* spp. cannot be directly used as bio-pesticides because of the bacterium’s inability to survive in soil for longer period. In view of this, alternatively, insecticidal

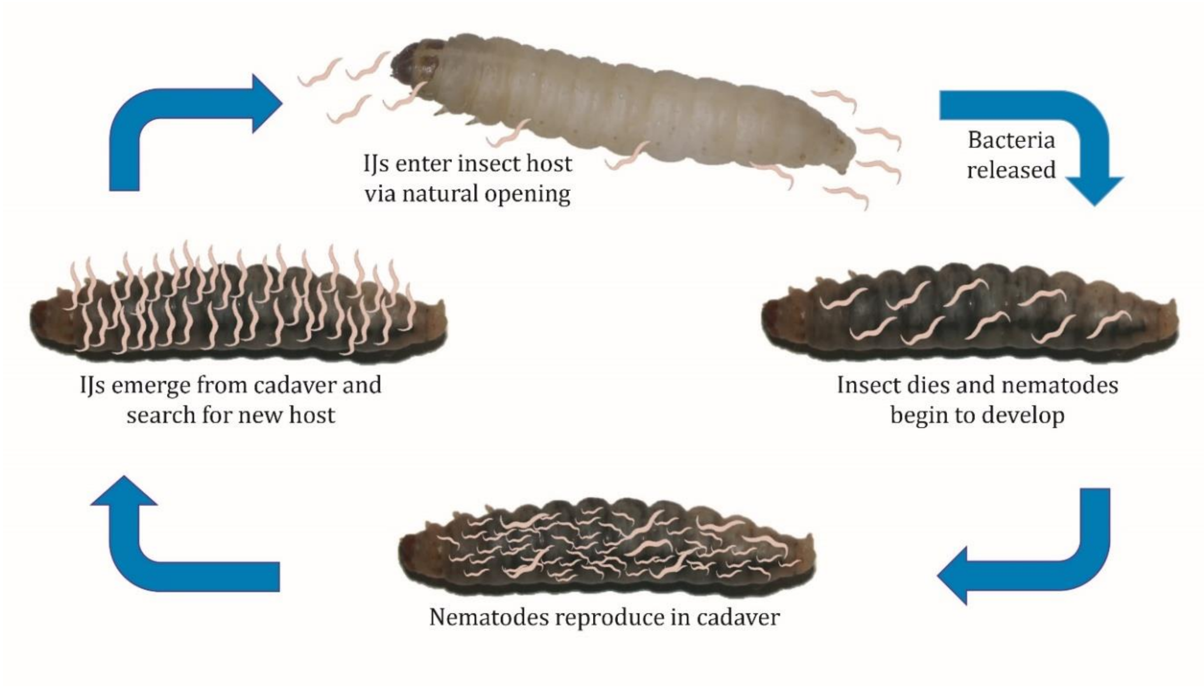

Fig. 1. Life cycle of EPN. IJs infect insects via an opening such as the anus, mouth, or spiracles, or by rupturing the cuticle. Once within the host, IJs shed their outer cuticle and begin consuming hemolymph that results in the regurgitation of symbionts. By 24-48 hours, the nematode–bacterium duo kill the host via septicemia or toxaemia. Next, the developing nematodes ingest the bacteria and bacterially metabolized liquefied insect tissues; nematodes mate together, reproduce and complete one or more generations before food resources get depleted. Bacteria re-associate with IJs that emerge from the depleted insect carcass in quest of new hosts.

property (specifically the toxin proteins) of these bacteria can be reproduced in other organisms such as bacteria or plant. In this direction, *Xenorhabdus* spp. and *Photorhabdus* encoded toxin genes and their secondary metabolites were studied by expressing those characters in heterologous hosts. For example, candidate toxins isolated from *Photorhabdus luminescens* when cloned and expressed in another bacterium, *Escherichia coli*, toxins displayed unprecedented pesticidal potential (Bowen *et al*., 1998). Subsequently, Morgan *et al*. (2001) reported the discovery of toxins from *Xenorhabdus nematophilus* and expressed its protein product in *E. coli* that conferred greater insecticidal property in the caterpillars of cabbage butterfly, *Pieris brassicae*. Since then a number of toxin genes were reported from both the *Xenorhabdus* spp. and *Photorhabdus* spp.

The whole genome sequencing of *Photorhabdus luminescens* strain TT01 and of *P. luminescens* strain W14exhibited that these bacteria constitute a wide spectrum of potential virulence factors such as high molecular weight toxin complexes (Tc), antibiotics, lipopolysaccharides, enzymes like proteases and lipases (ffrench-Constant *et al*., 2000; Duchaud *et al*., 2003). The Tc genes belong to a large gene family that harbour Tca, Tcb, Tcc and Tcd loci, with each locus exhibiting lethal effect on insect survival (Bowen *et al*., 1998; Blackburn *et al*., 1998; Waterfield *et al*., 2001). Other than that, various insecticidal toxins like Makes Caterpillar Floppy (Mcf) genes, *Photorhabdus* insect related (Pir) proteins, *Photorhabdus* Virulence Cassettes (PVCs), Photox, Pit, Txp40 etc. have showed oral and injectable toxicity against a wide spectrum of insect pests (Daborn *et al*., 2002; Duchaud *et al*., 2003; Brown *et al*., 2006; Yang *et al*., 2006). Waterfield *et al*., (2005) proposed that for full functional activity of Tc toxin, different Tc components (TcdA-, TcdB- and TccC-like) must complement each other. However, the catalytic activity or mode of action of these candidate toxins may vary in different strains of *Photorhabdus* spp. Additionally, India as a vast country constitutes diverse and numerous agro-ecological zones and accordingly insect pest incidence substantially differs. Therefore, exploring the toxin gene diversity from the Indian strains of *Photorhabdus* spp. can make an arsenal of choices to prepare the nation to combat global food scarcity.

Role of bacterial toxins in insect management:

Tc gene homologues were characterized in different bacterial genera such as *Xenorhabdus* spp., *Yersinia* spp. (YenTc in *Yersinia entomophaga* and *Y. pestis*), *Burkholderia* spp., *Pseudomonas* spp., *Serratia entomophila* etc. The genomic structure of Tc locus has also been compared with the *Salmonella* plasmid-borne virulence factor loci, spvA and spvB (ffrench-Constant *et al*., 2007). Nevertheless, the utility of these bacterial toxins is yet to be realized in insect pest management. On the contrary, Cry toxins have been extensively used in world agriculture. Given its enormous volume of research globally, current section depicts the utility of Cry toxins in pest management in a detailed manner in the following paragraphs.

Bacillus thuringiensis is a gram-positive, spore forming, rod-shaped soil bacterium ubiquitously found from a vast range of ecosystems and niches like soil, water, dead insects and plant tissues, insect-feeding mammals, and even from the human tissues having severe necrosis (Höfte and Whiteley 1989; Raymond *et al*., 2010; Palma *et al*., 2014). The bacterium was first discovered by a Japanese sericulture specialist Ishiwatari Shigetane from the infected silkworms, which he named as *Bacillus sotto*. Later, Ernst Berliner (1911) rediscovered the bacterium as a causal organism of *Schlaffsucht* disease of Mediterranean flour moth larvae in Thuringia state of Germany. The molecular phylogenetic analyses showed the bacterium as a close relative to *Bacillus anthracis* and *B. cereus* (Guinebretière *et al*., 2013). *Bacillus thuringiensis* (Bt) is uniquely known for producing insecticidal proteins that are active against different insect Orders and also some species from other animal Phyla (Table 1). During the sporulation phase, the Bt strains produce crystal (Cry) proteins called as delta-endotoxins (Palma *et al*. 2014). The toxin protein crystals get solubilized upon reaching the insect midgut *per os*, and disrupt the midgut cells being proteolytically activated. The toxins then interact with the specified receptors localized onto midgut cell surface leading to pore formation in the cellular membrane resulting in insect death (Badran *et al*., 2016).

The Cry proteins produced by the Bt bacteria are especially toxic against the Hexapod Orders Lepidoptera, Diptera, Coleoptera and Hymenoptera (Bravo *et al*., 2007). The proteins collectively comprise about 50 subgroups with 200 members;

Table 1. A summarization of Bt derived Cry and Cyt endotoxins affecting different animal hosts (Palma *et al*., 2014)

Protein components	**Active against**
Cry51A, Cry32A, Cry22A, Cry15A, Cry9A-9C,9E, Cry8D,Cry7B, Cry2A, Cry1A-1K,	Lepidopteran insects
Cry49A,Cry48A, Cry47A,Cry44A, Cry39A, Cry32B-32D, Cry27A,Cry24C, Cry20A, Cry19A-19B, Cry16A, Cry11A-11B, Cry10, Cry4A-4B, Cry2A, Cry1A-1C, Cyt2A-2B, Cyt1A-1B,	Dipteran insects
Cry55A, Cry43A-43B, Cry37A, Cry36A, Cry35A-35B, Cry34A-34B, Cry23A, Cry22A-22B, Cry18A, Cry14A, Cry9D, Cry8A-8G, Cry7A, Cry3A-3C, Cyt2C, Cyt1A-1B	Coleopteran insects
Cry22A, Cry5A, Cry3A	Hymenopteran insects
Cry11A, Cry3A, Cry2A	Hemipteran insects
Cry55A, Cry21A, Cry14A, Cry13A, Cry12A, Cry6A-6B, Cry5A-5B	Nematodes
Cry1Ab	Gastropods

and analyses of tertiary structures by X-ray crystallography suggested their structural similarity that aid in similar mode of action for all of them (Bravo *et al*., 2007; de Maagd *et al*., 2001). They contain a bundle of seven α-helices at the N-terminus (Domain I) where the hydrophobic central helix-α5 is encircled by six amphipathic helices responsible for pore formation and membrane insertion. Domain II is comprised of three numbers of anti-parallel β-sheets having exposed loops, and Domain III forms a β-sandwich. The exposed regions present in Domain II and Domain III primarily helps in receptor binding at the midgut membrane. More specifically, the Domain II of the Cry toxin shares structural similarity with carbohydrate binding proteins like vitelline, lectin jacalin, lectin Mpa; whereas Domain III shows similarity to the cellulose binding domains of β-glucoronidase, galactose oxidase, sialidase, 1,4-β-glucanase C, and carbohydrate binding domains of β-galactosidase and xylanase U (de Maagd *et al*., 2003). The crystal inclusions of the Cry proteins, upon being ingested, get dissolved in the alkaline midgut; thereafter the solubilized inactive protoxins yield 60-70 kDa active toxin proteins being cleaved by the proteases (Bravo *et al*., 2005). The activation of toxins involves enzymatic removal of specific stretch from the N-and C-termini (Bravo *et al*., 2007). The *cry* genes in most of the Bt strains encode for endotoxic CRY proteins that are located at plasmids. Additionally, Bt bacteria also produces cytosolic (Cyt) proteins that are insecticidal in action (Li *et al*., 1996). The Cyt proteins, unlike cry proteins, contain one α-β domain with two outer layers of α-helix hairpins wrapped around the β-sheet; and are structurally related to volvatoxin A2 toxin of the straw mushroom *Volvariella volvacea* (Palma *et al*., 2014). Bt strains have also been found to secrete some insecticidal proteins during vegetative developmental phaseinto the growing media (Schnepf *et al*., 1998; Estruch *et al*., 1996). The secreted proteins are mainly comprised of two classes as vegetative insecticidal protein (Vip) and secreted insecticidal protein (Sip) (Donovan *et al*., 2006; Estruch *et al*., 1996). The Vips and Sips contain specific signal peptides that are conserved, and cleaved after being secreted from the bacterium.

Bt derived spores and insecticidal Cry proteins are widely used to control the insect pests, particularly of Order Lepidoptera. The first Bt product, named as Sporeine, was commercialized in 1938 (Sanchis, 2011). Subsequently, several commercial preparations have been developed. To date, approximately 95% of the commercialized microbial bioagents are comprised of the Bt products (Schünemann *et al*., 2014). The majority of crystalline Bt based formulations are produced from Bt var. kurstaki HD1 (Cry1 Aa/Ab/Ac; Cry2Aa); Bt var. kurstaki HD73 (Cry1Ac); Bt var. aizawai HD137 (Cry1Aa/B/Ca/Da); Bt var. San Diego and var. Tenebrionis (Cry3Aa); and Bt var. israelensis (Cry4A/B; Cry11A; Cyt1Aa). These products are successfully used against several pests of agriculturally important crops like banana, cotton, citrus, corn, potato, tobacco, vegetables, and pasture (Schünemann *et al*., 2014). Further, transgenic crops expressing the Bt toxins provide a lucrative tool for integrated pest management. Notably, genetically modified tobacco was developed by the Belgian company Plant Genetic Systems (now under Bayer Crop Sciences) in 1985 was the starting point of Bt transgenics where the plants contained delta-endotoxin by expression of *cry* genes. Later, transgenic potato, corn, cotton, soybean, brinjal etc. have been developed expressing the Bt toxins; but not all of them have been given permission for open field cultivation (Abbas *et al*., 2018). Although the genetically engineered Bt crops reduce the pesticide usage and considerably drive off the destructive insect pest damage, they suffer from human and environment related controversies. Nevertheless, cultivation of Bt crops like Bt corn and Bt cotton showed no significant impact on the beneficial soil flora and fauna or non-target organisms (Mendelshon *et al*., 2003). Contrastingly, Abbas *et al*. (2018) showed that the Bt corn Mon810 produces specific toxins that interfere with human cell viability. In this line, In 2001, the US Environmental Protection Agency (EPA) reassessed the toxicity status of registered Bt corn with Cry1F, Cry1Ab; Bt potato with Cry3A; and Bt cotton with Cry1Ac on the non-target organisms including human and mammals; but the results revealed instability of the Bt in presence of human digestive fluids in gut and were degraded within seven minutes (Mendelshon *et al*., 2003).

The use of Bt formulations are relatively harmless to the humans and non-targets that provide considerable target specificity towards various insect pests. However, Bt-based transgenic plants and bio-pesticides are subjected to ethical considerations and safety issues, though researchers advocate for their cultivation based on laboratory and field studies. In this line, considerable dietary risk assessments, toxicological studies, allergenicity and digestibility studies, ecological risk assessments and environmental persistence studies should be conducted for safe use of the Bt-based products.

***Photorhabdus* toxins: New player in the game:**

Photorhabdus genus is considered as the ‘Pandora’s box’ for the discovery of novel insecticidal toxins, which could further be exploited as biocontrol agents either via transgenic mean or biopesticide formulation. Proteins identified in *Photorhabdus luminescens* including Tc, Mcf, PVC, binary toxins Pir, Photox etc. were found to be insecticidal when injected into the insect hemocoel. Only a few of them were found to be orally active. Additionally, a multitude of virulence factors were mined from the whole genome sequence of *P. luminescens* strains TT01 and W14.

a. Toxin complexes:

The Tc toxins are very large oligomeric tripartite toxins (collectively more than 1.4 MDa in molecular mass) that constitute three basic components such as A, B, and C. Putatively TcA functions as receptor binding molecule and toxin translocator, TcC confers the toxic enzyme activity, while TcB function as a linker between TcA and TcC components. Blackburn *et al*. (1998) reported the Tc toxin's high insecticidal nature against lepidopteran pest, *Manduca sexta*. Histopathology of Tca in *M. sexta* was also reported and Tc mode of action was very similar to other gut active toxins that cause blebbing of the midgut epithelium and eventually lysis of epithelium leading to gut leakiness.

Notably, the nomenclature of Tc genes is quite complex and very confusing. Tripartite Tc genes can be divided into three basic genetic elements, i.e. TcA, TcB and TcC types, that are located at four different loci or four pathogenic islands named as Tca, Tcb, Tcc and Tcd. Astonishingly, *P. luminescens* strain TT01 and W14 constitute a wide variety of Tc genes, i.e. 7 TcA- and TcC-type genes (Roderer and Raunser, 2019). Waterfield *et al*. (2001) showed that Tcs carry multiple copies of TcdA (encode tcdA1, tcdA2 and tcdA3) and TcdB (tcdB1 and tcdB2) genes which are homologous to each other. TcaA/B, TccA/B, TcbA and TcdA loci are quite identical to each other based on their encoded protein types, suggesting the predominance of Tc gene isoforms in *P. luminescens* genome (Sheets and Aktories, 2017; Fig. 2).

Albeit the ambiguity in Tc gene nomenclature, individual Tc gene components such as Tca, Tcb, Tcc and Tcd separately exhibited partial toxicity to different insects when heterologously expressed in *E. coli* (Morgan *et al*., 2001; Waterfield *et al*., 2005; Yang and Waterfield 2013). Bacterially expressed TcdA1 and TcdB1 (independently) conferred oral insecticidal activity when extracted from the supernatant of *E. coli* cells (Waterfield *et al*., 2001). Most importantly, Liu *et al*. (2003) attempted the transgenic or *in planta* expression of *Photorhabdus luminescens* TcdA1 gene in model plant *Arabidopsis thaliana*, that conferred insecticidal activity against tobacco hornworm *Manduca sexta* and southern corn rootworm *Diabrotica undecimpunctata*. A 63 kDa protein (named as Toxin B) isolated from *P. luminescens*strain W14 conferred oral insecticidal activity against *D. undecimpunctata* (Guo *et al*., 1999). Blackburn *et al*. (2005) demonstrated that Tca is an orally active toxin against the whitefly pest *Bemisia tabaci* and Colarado potato beetle *Leptinotarsa decemlineata*; the two important subunits of Tca, i.e. TcaAii and TcaAiii were revealed to be least toxic in nature. Based on this, they concluded that toxic activity of Tca is depends on itself and not on the other subunits such as TcaAii and TcaAiii. On the contrary, it has also been demonstrated that complete toxicity of Tc can be restored if its individual components are co-expressed together (Waterfield *et al*., 2001). The Tc toxin of *P. luminescens* carries its cytotoxic activity in the C-terminal domain of TcC that is hypervariable in nature. This cytotoxic domain is cleaved out from the Tc holotoxin

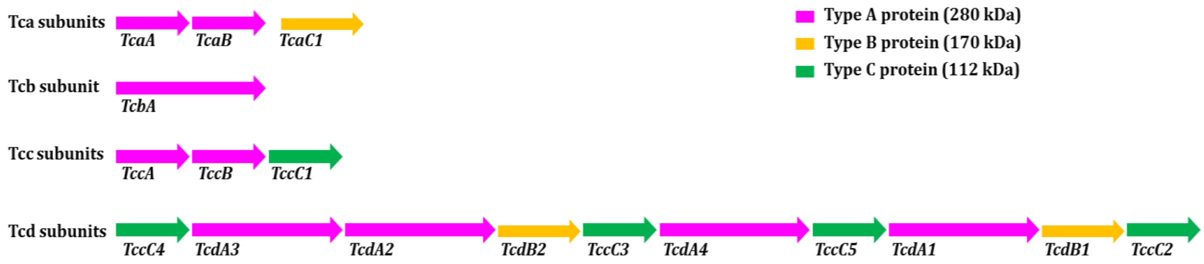

Fig. 2. The highly complicated nomenclature of Tc genes. Schematic representation of the structure and order of the *P. luminescens* Tc genes. Tc genes are found on four distinct pathogenic islands: Tca, Tcb, Tcc, and Tcd. The hue of a gene corresponds to the type of protein it encodes (Type A, B, or C). TcaA + TcaB are isoforms of the TccA + TccB locus and the TcdA or TcbA locus, respectively. Tc-binding and translocation are facilitated by Tca-like components. Tcb-related components act as a chaperone and connector between Tca and Tcc components. Tcc-like components contain the TccC3/C5 toxin enzyme. The TcaA/B, TccA/B, TcbA, and TcdA loci are nearly comparable in terms of the encoded protein type and sequence, indicating that Tc gene isoforms are prevalent in the *P. luminescens* genome.

(that comprises TcB-TcC complex) and liberated into the host cell cytosol during the Tc intoxication mechanism (Roderer *et al*., 2019).

A number of bacterial toxins target host actin molecules via ADP-ribosylation mechanism. Examples include binary toxins from *Clostridium botulinum* (C2 toxin), *C. perfringens* (iota toxin), *Clostridium difficile* transferase or CDT and vegetative insecticidal proteins (VIPs) from *Bacillus cereus*. *P. luminescens* TccC possesses ADP-ribosyltransferase (targets Rho proteins which are regulated by a GTPase cycle) activity and modifies actin molecule at Threonine-148 position. The translocation mechanism of Tc into target cells is assumed as injection syringe like phenomena, in which the toxic enzyme TccC5 causes actin polymerization that induces depolymerization of actin filaments and disintegration of actin cytoskeleton of the target host cells or insect hemocytes (Sheets and Aktories, 2017).

b. Makes Caterpillars Floppy (Mcf) toxins: Another important insecticidal toxin in *P. luminescens* genome is Mcf toxin. Mcf destroys the insect phagocytes, which are produced by insect immune reaction for killing the invading bacteria. Due to this, insect gut is badly damaged resulting in the floppy appearance of the larvae. Mcf protein mimics a BH3 domain that promotes apoptosis of the host mitochondrion. Mcf1-exposed cells exhibited fragmentation of host cell nucleus, host cell DNA laddering and activation of caspase-3 mechanism; all of that were indicative of cell death phenomena. Intriguingly, Mcf1 is detected in different genomic locations of different strains of *Photorhabdus* spp., implying that Mcf1 is a mobile genetic element in *Photorhabdus* genome. Conversely, Mcf1 is considered as an essential virulence factor as its homologues are found in all *Photorhabdus* strains, *Xenorhabdus* spp. and *Pseudomonas fluorescens* Pf-5 (Daborn *et al*., 2002; ffrench-Constant *et al*., 2007; ffrench-Constant and Dowling, 2014).

Waterfield *et al*. (2003) described a Mcf-like element in the genome of *P. luminescens* W14 strain and was named as Mcf2. Notwithstanding to Mcf1, amino termini of Mcf2 possesses a type III secretion system delivered HrmA avirulence gene or effector of *Pseudomonas syringae*. The carboxyl termini of Mcf1 and Mcf2 possesses repeat in toxin (RTX)-like domains, which are known to be secreted by the type I secretion system. The presence of both type I and type III export machinery signature in Mcf genes is quite intriguing and provide some clue about how this large protein is secreted out of the bacterial cell.

c. Photorhabdus Virulence Cassettes (PVC):

The PVCs are another toxin-encoding islands (found as tandem repeats of prophage-like loci) prevalent in the *Photorhabdus* genome. PVC loci can move around and between the bacterial genomes, indicating its mobile nature. PVC locus constitutes a conserved PVC element (encodes phage components including phage tails and baseplates) and a payload region (encodes ORFs corresponding to bacterial effectors such as sepABC of *Serratia entomophila*). PVC has homology to *Serratia* anti-feeding prophage, which is found on the pADAP plasmid that utilizes a type IV DNA conjugation pilus, thus indicating a common mechanism of horizontal transfer of genetic elements between *Serratia* and *Photorhabdus*. Transmission electron microscopic (TEM) images of recombinant PVCs showed that PVCs contain a phage tail-like particle that is quite identical to R-type pyocins (bacteriocins may deliver the payload region encoded toxin into the host cell). *Photorhabdus* PVC effectors caused rearrangement of actin cytoskeleton in the cells of mammalian tissue culture suggesting its possible mode of action and led to reduction of circulating hemocytes in *G. mellonella* upon hemocoel injection (Yang *et al*. 2006; ffrench-Constant *et al*., 2007). Vlisidou *et al*. (2019) demonstrated that Pnf (a Rho-GTPase) is a PVC needle complex associated toxin, which disrupts the cytoskeleton through transglutamination. Using TEM and Western blot it was shown that Pnf is necessary for delivery of PVC component in the host via the cell membrane.

d. Photorhabdus insect related (Pir) binary toxins:

Waterfield *et al*. (2005) identified two ORFs, *plu4092* and *plu4436* were closely located to similar loci *plu4093* and *plu4437* within the *P. luminiscens* TT01 genome and showed oral toxicity against the adult and larvae of different mosquitoes including *Aedes aegypti*, *Anopheles gambiae* and *Culex pipiens*. Initially, these assumed to be orphan genes derived via gene duplication event were named later as '*Photorhabdus* insect related' (Pir) proteins A and B which are very similar to δ-endotoxins from Bt. Intriguingly, the presence of a number of enterobacterial repetitive intergenic consensus sequences around the Pir locus is suggestive of horizontal acquisition of *Pir* genes in *Photorhabdus* spp. Li *et al*. (2014) showed that PirAB protein is a binary protein that contains of pirA and pirB genes in separate genomic locations and were expressed together (with the help of a linker) in *E. coli*. The purified PirAB fusion and PirA and PirB mixture exhibited toxicity to the fourth-instar *S. exigua* caterpillarin terms of cell shrinkage, blebbing of cell membrane, condensation of nucleus and fragmented DNA. Abnormalities in the gut epithelium with more swelling and shedding of apical membranes in *Plutella xylostella* was observed when insects were ingested with recombinant *E. coli* co-expressing Pir

A and Pir B proteins (Blackburn *et al*., 2006). Ahantarig and coworkers (2009) showed that PirAB fusion protein confers greater toxicity compared to either of the individual PirA and PirB components or PirA/PirB mixture.

e. Photox toxin:

Visschedyk *et al*. (2010) reported a novel mART (mono-ADP-ribosyltransferase) enzyme produced by *P. luminescens* called Photox. It's a 46-kDa toxin and have shown similar homology in catalytic region of the actin-targeting mARTs. It also caused cytotoxic effect in yeasts. Similar to TccC5 mode of action, Photox causes polymerization of host cell actin cytoskeleton by modifying the actin molecule at arginine-177 position. Nevertheless, Photox translocation mechanism in target host cell is yet to be determined.

f. XaxAB binary toxin:

Zhang *et al*. (2014) analyzed the *P. luminescens* TT01 genomic sequence, during which they found *plu1961* and *plu1962* ORFs are similar to XaxAB binary toxin from *X. nematophila* that are involved in apoptosis and necrosis in the cell lines of insect and mammal. Vigneux *et al*. (2007) discovered that *Xenorhabdus* toxin α-xenorhabdolysin (Xax) causes apoptosis in both mammalian and insect cells by acting as a cytotoxin.

g. Photorhabdus insecticidal toxin (Pit):

A novel toxin Pit was cloned from *P. luminescens* subsp. *akhurstii* YNd185 and expressed in *E. coli* (Li *et al*., 2009); the purified Pit protein caused pronounced mortality in model insect *G. mellonella* (LD_{50} - 30 ng/larva) and economically important insect *S. litura* (LD_{50} – 191ng/larva).

h. Txp40:

Brown *et al*. (2006) identified a new 42 kDa protein toxin, Txp40 from *P. luminescens* strain V16. This toxin was ubiquitously present in other 59 strains of *Xenorhabdus* spp. and *Photorhabdus* spp. Txp40 cytotoxic effect was observed in different insects including *G. mellonella*, lepidopetran pests *Plodia interpunctella*, *Helicoverpa armigera* and the dipteran insect *Lucila cuprina* due to the oral feeding of recombinant protein. The primary site of action of Txp40 is insect gut epithelium and additionally causes some damage to fat bodies.

The potential of *Photorhabdus* toxin for pest management in Indian scenario:

In India, the amount of money spent on pesticides is estimated around annual US$650, which is mainly intended to control bollworm and sucking pest of cotton. Dhaliwal *et al*. (2015) recorded that Indian farming is suffering from a huge loss of US$36 billion (16.8% in terms of percentage of yield decline) annually owing to insect pest incidence. It has also been recorded that percentage of yield loss of crops because of insect pest attack in major crops rose from 7.2% to 23.3% during the period between pre-green revolution era (1960s) and post-green revolution era (2000s). Additionally, due to the adoption of Bt cotton in Indian agriculture (occupying more than 95% of cotton producing areas), the yield loss due to insect pest is estimated at around 30% (Dhaliwal *et al*., 2010, 2015).

A majority of previously reported toxins from *P. luminescens* is insecticidal when injected into the hemocoel of test insects, and this is not desirable for further commercial deployment. In view of this, our laboratory is engaged in exploring the orally active toxins from Indian strains of *P. akhurstii* (a few of them recently elevated to novel Indian-subcontinent specific species and subspecies level such as *Photorhabdus hindustanensis* and *Photorhabdus akhurstii* subsp. *bharatensis*; Machado *et al*. 2021).

a. Txp40, PirA, TcaA, TcaB, TccC and TccC toxins from P. hindustanensis and P. akhurstii bharatensis:

Initially, a number of candidate genes were selected by *in silico* assay from the genome sequence database of *P. luminescens* strains TT01 and W14. A number of bacterial strains such as *P. hindustanensis*, *P. akhurstii bharatensis*, *P. akhurstii* strain IARI-SGGJ2 and *P. akhurstii* strain IARI-SGMS1 etc. were extracted from the IJs of *H. indica* nematode (collected from different geographical regions of India such as Meghalaya, Haryana, Gujarat and Maharashtra states) by culturing in nutrient bromothymol blue agar plates followed by 16S rDNA sequencing. The open reading frames (ORFs) or coding sequences of the toxin genes were cloned from different bacterial strains and transformed into *E. coli* cells. An extensive rapid virulence annotation (RVA) experiment was performed to screen out the potential virulence loci in these toxin candidates. For this, the recombinant *E. coli* clones containing toxin genes were intra-haemocoel injected (independently) into the fourth-instar larvae of model insect *Galleria mellonella*. Insect mortality data was recorded after 72 h of injection of six candidate toxins including Txp40, TcaA, TcaB, PirB, TccA and TccC. After identifying the potential ORFs that may correspond to bacterial virulence, these ORFs were cloned into a protein expression vector pET29a by restriction digestion method and subsequently transformed into heterologous host *E. coli* BL21(DE3) cells for functional investigation of these candidate toxins (Fig. 3).

Detailed *in silico* studies suggested the inter-strain gene sequence diversity at number of locations in the candidate toxins. This corroborated with the significant difference in the insecticidal activity of same protein toxins isolated from different bacterial strains, exemplifying the diverse gene pool of toxins in the Indian strains of *P. akhurstii* or *P. hindustanensis* leading to strain-dependent virulence in *Photorhabdus* bacterium (Dutta *et al*., 2020). It can be assumed that a single amino acid residue alteration might have significantly modified the biological activity conferred by the corresponding proteins. Notably, phylogenetic analysis of PirA showed its homologues in *P. akhurstii* and *P. luminescens* branch were nearer to a number of *Xenorhabdus* spp. such as *X. nematophila* and *X. poinarii*, but the same branched farthest from PirA homologues in *P. temperata*, *P. bodei*, *P. laumondii*, and *P. asymbiotica*. This exemplified the possibility of differential genomic location of PirA gene in several *Photorhabdus* species and thus, PirA is presumably mobile or transposable within the *Photorhabdus* genome (Dutta *et al*., 2020). TccC of *P. akhurstii* / *P. hindustanensis* contained YD repeat motifs, which are often located in the core domain of rearrangement hotspot (RHS) toxins, a part of polymorphic ABC toxins. Homologues of TccC were detected in 13 members of order Enterobacteriales including *P. luminescens*, *P. namnaonensis*, *P. khanii*, *P. temperata*, *P. thracensis*, *P.*

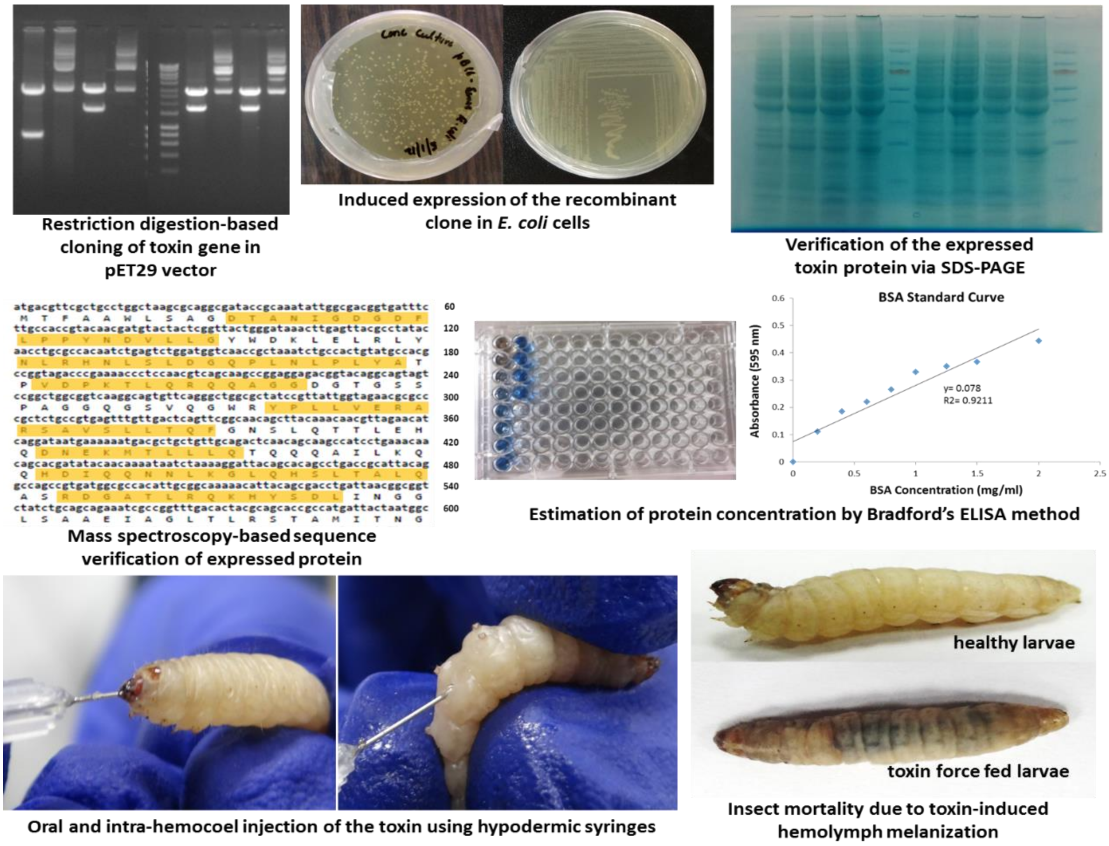

Fig. 3. Cloning, characterization and functional analysis of *Photorhabdus* toxins in *G. mellonella* fourth-instar larvae.

bodei, *P. laumondii*, *X. nematophila* and *X. bovienii*. On the contrary, TcaA of *P. akhurstii* / *P. hindustanensis* contained DNRLK/RE domain, which is predominantly found in *Bacillus* spp. Thus, TcaA homologues were detected in 16 members of Enterobacteriales order including a number of diverse genera such as *Yersinia*, *Erwinia*, *Burkholderia*, *Paraburkholderia*, *Aeromonas*, *Shewanella*, *Bacillus* and *Paenibacillus*. Similarly, TccA homologues were encountered in 15 members of Enterobacteriales order including *Burkholderia*, *Enterobacter*, *Salmonella* and *Granulibacter* (Dutta *et al*., 2020).

A *P. luminescens* Txp40 homologue was cloned from *P. hindustanensis*, *P. akhurstii bharatensis* and expressed in *E. coli* bacterium. The molecular weight of the purified, expressed protein was 37 kDa (encoded by an ORF of 1008 bp). The toxin protein conferred both injectable and oral toxicity in *G. mellonella* (Mathur *et al*., 2018). This was in stark contrast to original Txp40 protein (identified in *P. luminescens* strain V16) which was of 42 kDa in size and showed only injectable (no oral) insecticidal toxicity (Brown *et al*., 2006). Further, we demonstrated that Txp40 could confer both oral and injectable toxicity in agriculturally-significant insects such as cotton bollworm *Helicoverpa armigera*, tobacco cutworm *Spodoptera litura* and beet armyworm *S. exigua*. Haemocoel injection of the toxin in test insects (fourth-instar) resulted in the dose-dependent decline of total haemocytes (circulatory) and reduction in viability of haemocytes. Txp40 introduction in the hemolymph elevated the phenoloxidase (PO) activity to such extent that unrestricted melanization occurred ultimately leading to larval death. TEM-based histopathology study revealed the extensive damage in midgut epithelium indicating the entry of toxin from haemocoel to gut through leaky septate junctions or catalytic action of the toxin molecule (Shankhu *et al*., 2020). A domain conservation study (that includes multiple threading and segment assembly technique) of TcaB revealed its potential homologues as a binary toxin of *Yersinia entomophila* (YaxAB), a binary toxin of *Xenorhabdus nematophila* (XaxAB), a cytotoxin of *Vibrio cholerae* (makA) and ADP-ribosyltransferase enzyme of *Legionella pneumophila* (Shankhu *et al*., 2020).

A C-terminal domain of TcaB toxin (1713 bp) was cloned from *P. akhurstii bharatensis*, *P. akhurstii* IARI-SGMS1 and expressed in heterologous host *E. coli*; the molecular weight of the purified, expressed protein was 63 kDa. TcaB toxin exhibited both injectable and oral toxicity in beehive pest *Galleria mellonella* (Mathur *et al*., 2019). Upon hemocoel injection, TcaB conferred pronounced cytotoxicity and immunomodulatory activity (in terms of increased phenoloxidase activity in the haemolymph). Hemocytes displayed typical morphological aberrations including cell shrinkage, blebbing of cell membrane, nuclear condensation and degradation, ultimately leading to apoptotic cell death (Mathur *et al*., 2019). TcaB constitutes several functional domains including a zinc metalloprotease / metazincin domain (42-170 aa; HEXXH signature) that is the determining factor of bacterial virulence (indicative of TcaB catalytic activity determinant), a transcription regulator motif (108-178 aa) overlapping the metazincin domain and a receptor binding domain (283-565 aa). TcaB domain conservation analysis showed its notable homologues as TcdA1 toxin of *P. luminescens* (Protein Data Bank accession number: 1VW1), Tc toxin of *Yersinia entomophaga* (6OGD), Cry2A toxin of *B. thuringinesis* (1I5P) and a crystal protein of *Bacillus* sp. (1J0M). The oral delivery of TcaB caused extensive damage in the gut epithelium of *G. mellonella* fourth-instar larvae during 6 to 24 h of incubation period; a gradual decline in gut homeostasis led to movement of TcaB into the hemocoel. Consequently, upon migration from alimentary canal to body cavity via leaky gut, TcaB conferred an unprecedented cytotoxicity and immunostimulatory activity in the *G. mellonella* haemocoel after 6 to 24 h of oral delivery (Santhoshkumar *et al*., 2021). The gut-active nature of the TcaB toxin was also demonstrated in the agriculturally-important insects including fall armyworm *Spodoptera frugiperda*, *S. litura* and *H. armigera* (Dutta *et al*., 2021a; Fig. 4).

b. Galtox from P. hindustanensis and P. akhurstii bharatensis:

During the search of potential Photox toxin variants in the genomes of seven *Photorhabdus* symbionts from *Heterorhabditis* nematodes of Indian subcontinent, a novel protein toxin Galtox (35 kDa in molecular weight) was identified serendipitously. Unlike of Photox (46 kDa toxin), Galtox did not confer oral toxicity in *G. mellonella* and do not encode the characteristic actin-targeting mono-ADP-ribosyltransferase (mART) domain. Galtox conferred rapid insecticidal activity via intra-hemocoel injection with LD_{50} values of 3.1-31.2 ng per g of *G. mellonella* body mass. Domain conservation analysis indicated the homology of Galtox with other

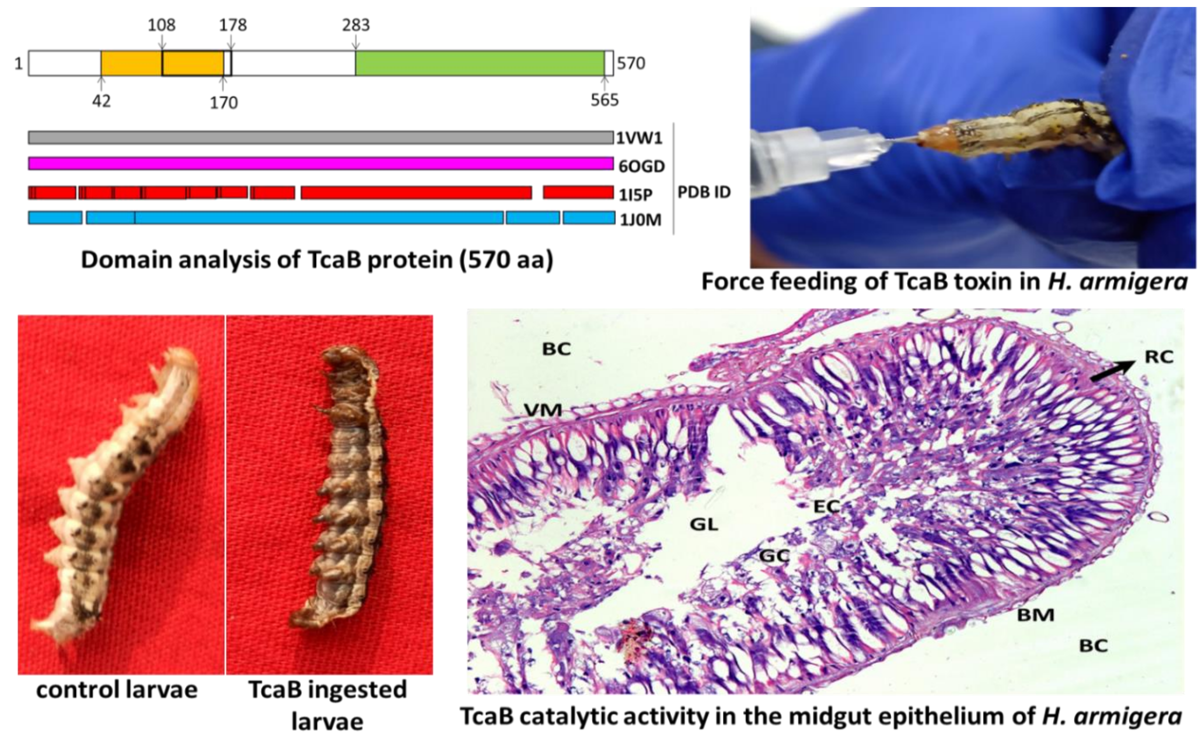

Fig. 4. TcaB induced gut leakiness in *H. armigera*. TcaB contains a catalytic domain (indicated in yellow box), a transcriptional regulator motif (empty box) overlapping the catalytic domain and a receptor binding domain (green box). TcaB conserved domains are homologous to a number of bacterial toxins (PDB accession numbers: 1VW1, 6OGD 1I5P and 1J0M). Upon oral admistration of TcaB in the fourth-instar larvae, TcaB caused degeneration of epithelial cells (EC), goblet cells (GC) and regenerative cells (RC) which sloughed off into the gut lumen (GL). Rupture in the basement membrane (BM) lining and visceral muscle (VM) putatively led to escape of TcaB to the body cavity (BC) or hemocoel.

bacterial toxins such as α-Xenorhabdolysins (Xax) pore forming toxin complex of *Xenorhabdus nematophila*, BteA cytotoxin (exported via type III secretion system) from *Bordetella* spp., LegC3 effector from *Legionella pneumophila* and cysteine proteinase staphopain B (SspB) from *Staphylococcus* spp. (Ahuja *et al*., 2021).

c. Putative gut receptors for orally active toxins:

After extensive bioassay and histopathology analyses, we identified two gut-active toxins, i.e. Txp40 and TcaB from the Indian strains of *Photorhabdus* spp. Using an *in silico* protein-protein interactor study, it was predicted that Txp40 may dock with different gut receptor proteins from *H. armigera* such as cadherin (CAD), ATP-binding cassette transporters subfamily C (ABCC), aminopeptidase N1 (APN1) and alkaline phosphatase (ALP), that are putatively located in the cell membrane of midgut epithelial cells of *H. armigera*. The electrostatic bonds (interaction energy up to 575 $kJmol^{-1}$) between Txp40-CAD, Txp40-ABCC2, Txp40-APN1 and Txp40-ALP complexes were 6, 4, 1 and 9 in number, respectively; the hydrogen bonds (interaction energy up to 150 $kJmol^{-1}$) between identical ligand-receptor complexes were 15, 16, 14 and 22, respectively (Shankhu *et al*., 2020). Similarly, TcaB docked with CAD, ABCC2, ALP and APN1 receptors of *G. mellonella* via numerous hydrogen bonds, salt bridges and pi-alkyl bonds. The docking scores (calculated by total interaction energy in terms of distance-based pair potential per residue, where negative score suggests greater energy) for TcaB-CAD, TcaB-ABCC2, TcaB-APN1 and TcaB-ALP complexes were obtained as –527.54, –220.88, –80.54 and –188.48, respectively, implying the superior thermodynamic stability of the docked models (Santhoshkumar *et al*., 2021; Fig. 5). Further, TcaB also docked (binding energy of ligand-receptor complexes rangedbetween –50.14 to –72.34 kcal mol^{-1}) with candidate gut receptors from *S. frugiperda*, *S. litura* and *H. armigera* (Dutta *et al*., 2021a). Recently, using ligand blot, ELISA and RNAi studies it has been experimentally proved that *P. akhurstii* TcaB binds with membrane proximal domain of CAD protein in the midgut epithelium of *G. mellonella* (Dutta *et al*. 2021b).

Conclusion and future directions:

Due to the existence of diverse agro-ecological zones in the vast country like India, Indian farming system suffers heavily from the incidence of a broad spectrum of insects and pests. Development of insect resistance (in Western corn rootworm *Diabrotica virgifera virgifera*, corn earworm *Helicoverpa zea*, *H. armigera*, *S. frugiperda*, pink bollworm *P. gossypiella* etc.) has become an alarming concern because of the continuous monoculture of transgenic Bt plants (Bt maize, Bt cotton etc.). Therefore, exploring the untapped diversity of *Photorhabdus* toxins originated from the Indian strains may strengthen the pest management repository and will provide an alternative to Bt-dominated pest management tactics. The efficiency of the EPN or its symbiont bacterium as biocontrol agent rely on an optimum and narrow spectrum of moisture and temperature in soil. Additionally, EPN's application for foliar pest management has been limited because of IJs' sensitivity to ultraviolet radiation, desiccation and extreme temperatures. In view of this, efforts were undertaken to replicate the insecticidal activity of *Photorhabdus* toxins in heterologous microorganism such as *E. coli*. To date, a number of toxin candidates were characterized from *P. luminescens* W14 and TT01 (ffrench-Constant *et al*. 2007; ffrench-Constant and Dowling 2014; Sheets and Aktories 2017). Nevertheless, the sequences, conserved signatures and mechanism of action of the toxin candidates we characterized from *P. hindustanensis* and *P. akhurstii bharatensis* differed greatly when compared with the identical candidates from *P. luminescens* W14/TT01. Notably, bacterial genomes exhibit a greater degree of genetic/molecular variation compared to other organisms and astonishingly, strains corresponding to the identical species may confer a considerable 3 to 4% difference in their genomes (Ochman *et al*., 2000; Konstantinidis *et al*., 2005).

Our investigations unravel the structure and function of orally active *Photorhabdus* toxins in different Indian strains of *Photorhabdus* spp. By carrying out the immunological and toxicological investigations, we propose that *P. akhurstii* TcaB and Txp40 can negatively alter host insects' innate defense responses and function as a cytotoxin. Additional experiments are required to the establish the probable immunomodulatory property of TcaB and Txp40. *P. akhurstii* TcaB

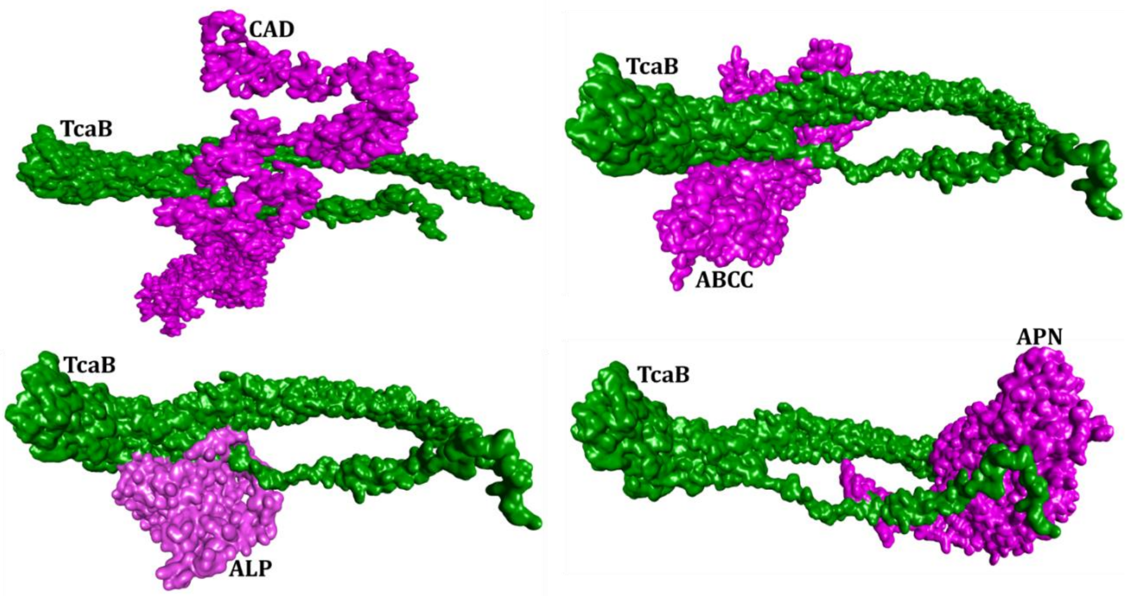

Fig. 5. Simulated binding site interactions of *P. akhurstii* TcaB and *G. mellonella* midgut proteins – CAD, ABCC2, ALP and APN1. Numerous hydrogen bonds, salt bridges and pi-alkyl interactions were detected in TcaB-receptor interface. Protein-protein interactor analysis was carried out in PatchDock webserver (https://bioinfo3d.cs.tau.ac.il/PatchDock/) and docked models were visualized via Discovery studio (v. 4.2). Best models were screened out according to the geometric shape complementary score and minimum energy potential between interacting molecules.

homologues were detected in a broad range of bacterial genera suggesting that Tc genes are indispensable in bacterial lifestyle and their evolutionary processes. TcaB and Txp40 were predicted to be exported from bacterial cell by the bacterial type III secretion system suggesting their effector-like nature. Owing to their smaller molecular mass (63 and 37 kDa), TcaB and Txp40 are suitable candidates for incorporating in molecular breeding of crops for insect resistance. It is worth mentioning that a plant-optimized version of a smaller subunit of *P. luminescens* Tc holotoxin, i.e. TcdA (transcription of the target was enhanced by adding 5′ and 3′ untranslated region (UTR) flanking sequences of *Nicotiana benthamiana* osmotin gene) was transgenically expressed in *Arabidopsis thaliana* that conferred substantial resistance to the larvae of *M. sexta* and *D. undecimpunctata* (Liu *et al*., 2003). There exist many unresolved queries related to the binding of toxin protein to target host cells, their delivery into, and the action of the biologically active components in the host cells. Any successful use of *Photorhabdus* gut-active toxins in agriculture warrants some basic research on the target receptors in the insect midgut. In view of this, the following approaches are advised to identify the potential gut receptors of TcaB/Txp40 protein in different test insects:

- A detailed bioinformatics analyses of CAD, APN, ALP and ABCC across the insect genera to predict toxin binding domains,
- Protein expression of truncated receptors and real-time receptor-ligand binding analysis by western blotting and ELISA,
- RNA/DNA isolation, cloning and characterization of the receptors from different test insects,

- CRISPR-Cas9/RNAi-based knockout/knockdown of selective toxin binding domains in test insects and behavioral/biochemical assays of mutant insects upon oral administration of TcaB/Txp40 toxin,
- RT-qPCR-based comparative expression profiling of receptors in control and mutant insects.

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Tête-à-tête with Dr. Chandrasekharaswami Adiveyya Viraktamath

GREAT TAXONOMIST, TEACHER AND ENTOMOLOGIST PAR EXCELLENCE WHO SERVED ENTOMOLOGY FOR 60 GLORIOUS YEARS

Dr. Chandrasekharaswami Adiveyya Viraktamath, fondly called CAV and Chandra, was born on 31 January 1944 to Sri Adiveyya, an employee in the revenue department and Smt. Neelambika at Byadagi, in northern Karnataka. Dr. Viraktamath had his school education in different places due to his father's transfers and joined degree course, B. Sc. in Agriculture at the College of Agriculture, Dharwad in 1961. During the College, he won ASPEE Gold Medal in Plant Pathology for securing highest marks. After completion of B. Sc. (Ag) degree, Dr. Viraktamath joined M. Sc. (Ag) at the College of Agriculture, Hebbal, Bengaluru in 1966 with Entomology as specialization. During this time, Dr. Viraktamath got influenced by Drs. H. M. Harris and J. H. Lilly, visiting Professors under Ford Foundation Progamme, who taught Insect systematics and Physiology, respectively. In due course, Dr. Harris impressed upon Dr. Viraktamath to take up insect taxonomic studies and he used to call Dr. Viraktamath as Vakma and also as Mutt by those who found difficult to pronounce his long name. Soon after M. Sc. (Ag) programme, Dr. Viraktamath joined as Research Assistant at Hebballi farm, Dharwad and then as Instructor at College of Agriculture, Dharwad. Later he joined Ph. D in 1970 to pursue doctoral degree in Agricultural Entomology. Dr. Viraktamath visited Oregon State University, Corvallis during 1970-72 and took up leafhopper taxonomy as his research work under the mentorship of Dr. Paul W. Oman, a renowned leafhopper taxonomist at that time. There Dr. Viraktamath worked on Old World Agalliinae leafhoppers examining a large number of species present in Oman's laboratory and other Museums besides collections from fellow entomologists pooled from all over the world. He had also visited California Academy of Sciences, where he indulged in sorting the Old World collection of leafhoppers and borrowed the agalliine leafhopper specimens to work upon. After 18 months of his stay in Oregon State University, Dr. Viraktamath returned to the College of Agriculture, Dharwad as a learned taxonomist and began search for the types of agalliine leafhoppers of the Indian subcontinent deposited in various Museums in addition to his fresh collections from all over India.

Dr. Viraktamath moved to College of Agriculture, Bangalore in the rank of Assistant Professor during July 1973, where he intensified his research balancing the workload of teaching both at undergraduate and post graduate levels. He continued enriching his collections of leafhoppers with an oversized 5.5 ft long collection net designed by Dr. Oman, which was longer and heavier than the usual normal net. In all outdoor field collections and expeditions, Dr. Viraktamath's wife, Smt. Lalita too took part and had cheerfully cooperated and helped Dr. Viraktamath in sorting, pinning and mounting of the leafhopper collections.

Dr. Viraktamath's first paper on leafhopper taxonomy was published in 1972 with a description of a new species, *Austroagallia* from Galapagos Islands. Subsequently he published about 15 papers in leafhopper taxonomy before his final submission of Ph. D thesis entitled, "A generic revision of the Old World Agalliinae (Homoptera: Cicadellidae)" in 1982. Dr. Viraktamath, notably recognized by his work, was invited to present a paper and chair a session in the "First International Workshop on biotaxonomy, classification and biology of leafhoppers and planthoppers (Auchenorrhyncha) of economic importance" held at London by Commonwealth Institute of Entomology" during 4-7 October 1982. Later Dr. Viraktamath strengthened the leafhopper taxonomy in India with his vast collections and regular visits to Natural History Museum, London and once to National Museum of Natural History, Washington DC.

Dr. Viraktamath had published 208 research papers in peer reviewed journals, which include 112 papers on Cicadellidae with 44 revisionary studies and 11 reviews of various taxa besides description of a new tribe and several new genera and species. In all, 56 new genera and 424 new species have been described by Dr. Viraktamath and his collaborators. He has contributed to six edited books and several book chapters.

Dr. Viraktamath has been a role model and inspiring, teacher and mentor to several students, researchers and scientists. Though he is very strict with respect to the learning and practicing taxonomy, he has very deep and sympathetic concern towards students and he is the most sought after teacher. Dr. Viraktamath had a greatest concern towards insect collections and his deep commitment and dedication has shaped up a well curated modern Museum at the Department of Entomology, University of Agricultural Sciences, Bengaluru, which houses more than 3,50,000 specimens. Dr. Viraktamath has given innumerable identification services to the students, researchers, farmers, scientists, amateurs, Institutions (around 275), etc., who benefited immensely. He has guided 29 M. Sc and 14 Ph. D students in Agricultural Entomology, of which majority (27) studied on the taxonomy of different groups of insects.

On reverence and contribution to leafhopper taxonomy, several insect genera and species are named after him by many of his students and researchers. Around 22 species of leafhoppers are named in his honour by leafhopper taxonomists across the world. His drawing techniques are mentioned as notable and enviable by his students. With greater reverence, admiration, affection and gratitude, several taxa of Lepidoptera, Hymenoptera and Coleoptera have been named after him. In recognition of his expertise and experience, Dr. Viraktamath was invited as a guest faculty to Northwest A&F University, Yangling, Shaanxi Province, China by Dr. Zhang Yalin to assist postgraduate students in leafhopper taxonomic studies.

Dr. Viraktamath has received several awards and recognitions, of which the latest award by Govt of Karnataka, the Prof. CNR Rao-KSTA Lifetime Achievement Award for the year 2022 is the unique in recognition of his contribution in the field of Science and Agriculture.

Dr. Viraktamath working relentlessly on leafhopper taxonomy at the age of 78

"Indian Entomologist had the privilege of interviewing Dr. C. A. Viraktamath, living legend of Insect Taxonomy. Dr. Kolla Sreedevi (KS), Associate Editor of IE, interacted with Dr. Viraktamath (CAV) and the excerpts of the discourse are presented below."

KS: Sir, can you briefly let us know the driving factors that made you take Agriculture course?

CAV: Firstly, it's my parents who motivated me to take Agriculture course. My mother supported and encouraged all of us - six brothers and two sisters with good education and her blessings made us settle in good profession. My father was the guiding force, who took me to the beautiful campus of the College of Agriculture, Dharwad (Now, University of Agricultural Sciences, Dharwad) immediately after my one year PUC. I got impressed by seeing the College and decided to take Agriculture as a career though there was a chance of opting for a medical course. That's my first turning point. After joining the Agricultural College, Dharwad, the best of its kind, the Principal of the College, Dr. S. W. Menasinakai motivated us to study well and the staff members taught us well, that helped me to develop deep interest in Agriculture.

KS: What prompted you to zero-in on Agricultural Entomology as your specialization in M. Sc (Agri)?

CAV: Ours was the first batch of postgraduate courses in the young University. When I graduated from College of Agriculture at Dharwad in 1966, 6-7 classmates from Dharwad campus decided to join the PG course together. During that time, there were only five seats in each discipline out of which, two were reserved for In-service candidates. So, left out with only three seats open for each discipline, we all decided to divide and take the different disciplines to avoid completion among ourselves and I was fortunate to get Entomology and joined the course at College of Agriculture, Hebbal, Bengaluru. This is another turning point of mine to get into M. Sc. (Agri.) in Entomology.

KS: We would like to know the motivation behind pursuing taxonomy as your specialization?

CAV: Since the University had just started the PG courses, the entire course curriculum was drafted by the University with active participation of foreign and Indian experts following Land-grant system and at that point of time, Ford Foundation played an important role by deploying experts from abroad to strengthen teaching and research in the young University. Dr. H. M. Harris, a well-known Hemiptera taxonomist who worked on the American damsel bugs (Nabiidae) was helping Entomology department and offered a course in Insect Taxonomy in which I scored the highest marks. Dr. Harris identified my interest in Taxonomy and encouraged me to work on insect taxonomy and suggested to take up hemipterans for my study and in fact wanted me to work on aquatic hemipterans to begin with. This is another major turning point in my professional career to take up insect taxonomy as my specialized area of research.

KS: Now, we are interested to know your inclination to leafhoppers among several groups of insects for taxonomic studies?

CAV: When I was motivated to work on hemipteran taxonomy, there was wide spread occurrence of two diseases of ragi, Ragi streak virus and Ragi mottle streak virus both were suspected to be transmitted by leafhoppers, which attracted the attention of entomologists. However, there were no specialists available in India who could identify these leafhoppers and thus Dr. Harris suggested me to work on leafhopper taxonomy. That's the beginning of my interest in leafhoppers. Dr. Harris helped me in initial collection and curation of leafhoppers. I was fortunate as this period coincided with the publication of Z. P. Metcalf's "General Catalogue of the Homoptera", which drew more attention and interest. After my PG studies, I joined as Research Assistant at Hebballi farm, Dharwad and later as Instructor at the College of Agriculture, Dharwad. During this period I was selected for my doctoral programme under Ford Foundation Fellowship. This was a hybrid programme between University of Agricultural Sciences and any of the Universities in the US. The programme included doing part of doctoral programme in the chosen American University (mostly course work) and completing the remaining requirements of the research in the University of Agricultural Sciences, Bangalore. I was selected to work on the taxonomy of leafhoppers at Oregon State University, Corvallis during 1970-72 to work under the guidance of Dr. Paul W. Oman, one of the world leaders of leafhopper taxonomy at that time. I learnt alphabets of leafhopper taxonomy under his able guidance. After returning to College of Agriculture, Bengaluru, it was Dr. G. P. Channabasavanna, who guided me to complete my doctoral programme at the University in Bangalore. I am deeply indebted to all three, Dr. Harris, Dr. Oman and Dr. Channabasavanna for motivating me to pursue insect taxonomy as a passion and career.

KS: May we know the challenges you have faced in leafhopper taxonomic research at initial stages?

CAV: My research work for my doctoral programme was Revision of the Old World Agaliinae, at that time a subfamily in the family Cicadellidae. There was hardly any collection at the College. I had to make my own collections and borrow material from various museums in the world. I had to either borrow or personally visit European museums including the British Museum of Natural History, London to examine types. All these were challenges at that time and I started touring extensively in India to build up leafhopper collection from different parts of India. I was very fortunate as most of the museums did cooperate to send the material and also help in the study of types as Dr. Oman introduced me to these museums and all the leading leafhopper workers in the world at that time. Initially I used Dr. Coleman's old microsope to draw the habitus of the specimens and utilized prism or mirror type *camera lucida* to draw the characters. An ordinary student microscope was used to study the male genitalia of specimens. Later, the Leitz and Leica microscopes with *camera lucida* were procured and used, which made the microscopic studies much easier.

KS: What's your piece of advice or direction or guidance to the young researchers in Entomology?

CAV: Good training and mentorship is extremely important for any researcher, let it be any field of Entomology. Our batch was very well trained by Dr. Channabasavanna and Dr. M. Puttarudraiah with good grounding in basic aspects such as insect morphology, anatomy and physiology. I still remember the type of training we got from Dr. Channabasavanna. Having a passion for acarlology in addition to different fields of entomology, he used to examine the mounted specimens under oil immersion spending hours examining the tiny eriophyid mites. He trained us how to look at various minute structural details, microsetae, macrosetae, sensilla etc, under the microscope and spot the characters. For my Masters programme, I worked on rice case worm. In addition to biology and ecological studies, I was encouraged to study morphology, anatomy, chaetotaxy of larvae, pupae and adult rice case worm. Dr. Channabasavanna personally used to sit with me and other postgraduate students and help us to identify different types of structures and setae. This personalised training and guidance helped us to imbibe the nuances of good teaching and research, and pass it on to our students. I see now-a-days, the training is diluted, priorities are shifted and the present teaching and research system needs a major overhauling.

KS: What is the specific advice to the taxonomists especially the budding taxonomists?

CAV: First of all, passion, focus and dedication with lot of patience are very important to all taxonomists. For young taxonomists, my sincere advice is to break the barriers, correspond with all the leaders in the group, make good collection from diverse areas, develop cordial relations with the fellow taxonomists, discuss and cooperate. Insects do not recognize political boundaries. Therefore, world should be your playground not a restricted area of your state or country. One has to examine the type (especially primary types- holotype, syntypes, lectotype and neotype) or get help from those scientists who have examined the types. Indian Institutes lack good collection, unless and until collections representing world zoogeographical regions are made available, taxonomic studies will not progress much. I can sum up the three essential things for good taxonomic work:

1. Extensive literature
2. Good equipment like microscopes, for illustrations, camera for photography
3. Exhaustive collections and
4. Passion to work on taxonomy

To this list I would add a good molecular laboratory facility or a healthy collaboration with a molecular biologist.

KS: You are admired both as an excellent teacher and acclaimed taxonomist. So, how did you balance both teaching and research and which will you weigh more Sir?

CAV: Both are important, one has to strike a balance between Teaching and Research. When I joined as an Instructor, more weightage was given to teaching as there were less staff members. Less time was devoted to research in a day. But I weighed both jobs equally and strived hard to carry them hand in hand. I enjoyed both and derived tremendous satisfaction from both the jobs. Teacher influences and inspires young minds, the future scientists, teachers as I have emphasized earlier, I was influenced by Dr. Harris to take up insect taxonomy and Dr. Oman to take up leafhopper taxonomy. Therefore, both teaching and research, are important in their own way.

"Passion, focus and dedication with lot of patience are very important to all taxonomists"

KS: Can you narrate one incidence where you could bring change in the teaching methodology for the youngsters or young teachers to think innovatively?

CAV: The practical classes in Systematics course used to be dealt with earlier in a different way where the Instructor use to give the students, identified specimens and asks the students to examine them and draw the characters. When I took over as In-charge of the course, I changed this system. Maintenance of a record by the students was done away with. The students were given unidentified specimens of the group which they have to study and identify the family by running through the identification keys independently. I helped them with understanding the characters whenever they had problem. By this technique, when the student identifies the specimen correctly to a family he is satisfied and happy and remembers the characters better. This also makes the student self-confident in identifying the specimens at least to family level independently with the help of available keys.

Dr. Kolla Sreedevi (KS) in conversation with Dr. Chandrashekharaswami Adiveyya Viraktamath (CAV)

KS: Apart from taxonomy, have you worked on any other areas Sir?

CAV: Yes, I have worked on Economic Entomology also. A few projects to name are a) management of red headed hairy caterpillar in Pavagada, Karnataka; b) Mexican beetle, *Zygogramma bicolorata*, where we established that it's not a pest of sunflower; c) management of Gherkin pests, where we provided a schedule of operations for the management of its pests and diseases. I have also guided students on studies on insect biology and pest management, etc.

KS: Taxonomists often fear of late publications, your view on this?

CAV: Initially taxonomists fear for the work and the publications. Often, it's a starting problem and once it's initiated, it will be carried out with ease. I can say it's like going for a swim; initially one will fear to enter water, once he/she jumps, he/she enjoys the swimming.

KS: You are emphasizing the insect collections as one of the important requirements, can you please elaborate?

CAV: In India, insect collections are very poor and the existing collections are also poorly maintained, whether types or non-types. There is hardly any financial support for upkeep of specimens. Institutions where insect collections are there should know that the insect collections are the property of science; they are the heritage of the nation throwing light on the biology, ecology and evolution of organisms. Insect collections are built up by the passionate individuals often risking their own life during collections in the field, forest and unhygienic conditions and from inaccessible terrains, infested with vectors of diseases, etc. When I started my work on leafhoppers, there was hardly a box of collection, where the specimens were not in proper condition and most of them were wrongly identified. I, then started collecting the specimens from all over the country to rebuild the collection. My collection now comprises of about 70-80% of known Indian fauna of leafhoppers, which is properly maintained at UAS, GKVK campus, Bengaluru. In the Museums, the insect collections should show good representation from various geographical regions of the country and also the World. I deem that the collections are 'Property of the Nation' that depicts the diversity. In this era of rapid extinction of the species, the species should be first preserved before it goes extinct, the species may go extinct any time. The documentation derived from the collections will help in identifying and establishing the pest free areas from the international trade perspective. In fact, the collections are more valuable than the libraries. It has multiple benefits to the individuals, Institutions and the Nation. So, the collections are of utmost importance.

"Insect collections are Property of the Nation"

KS: It is generally felt that the taxonomists have become a rare or scarce taxa, what can be the measures to attract more people to this field?

CAV: Good foundation on basic aspects of science to be taught in the Universities as part of curriculum is very essential. Teachers' influence will be more in attracting the students towards a particular discipline. Administration should give more attention. Exclusive funding towards the taxonomic research is essential as the surveys and collections are involved. Networking and collaborative projects need to be enhanced. All these should be addressed to produce more taxonomists. In many areas of economic entomology there is a need for a specialized taxonomist, for example biological control, pest management, vector control, ecological studies, behavioral studies, quarantine, etc. and this necessity is often not realized and as a result the project outcome is not as good and sustainable as it would have been otherwise. Therefore, there is a necessity of creating job opportunities for taxonomists in these areas which will definitely attract young and innovative minds to insect taxonomy.

KS: What are the ways and means to make taxonomy attractive and also to see the growth of taxonomic field?

CAV: Most importantly, the job opportunities have to be provided. As there are more encounters of invasive species in India in recent times, the taxonomists to be trained on each specific groups and have to be deployed to address the issues with proper identification. Proper recognition has to be given to taxonomists and encourage more people to take up taxonomy and become specialists in each group of insects. Also, more attention and focus to be given on the development of second line of taxonomists.

KS: The taxonomic studies and research now-a-days is advancing faster, the literature is accessible, requirements are specified and set, equipments are procured, in this context, may I have your opinion on the quality of research, whether improved or deteriorated?

CAV: I feel it's improving with the digital imaging and line drawings with the help of software like Adobe photoshop, etc., and also improvised image capturing systems. The descriptions are full pledged and detailed, but these are all with the serious taxonomists. Those taxonomists, who are serious, working with passion and dedication are enumerating the morphological characters with a deeper insight into the taxonomic characters and illustrating the same, which can form a good foundation for the new generation or beginners to understand and start their work. On the other side, with non-taxonomists, who depend heavily on internet and proceed to document without proper research on the validity of information available, are coming out with wrong identifications, descriptions and poor quality illustrations. Often they end up in reporting species that are not present in the country, which poses a major threat for the international trade and un-necessary precautions at the port entry against pests or vector species (misidentified) that do not occur in the country.

KS: May I have your views on molecular taxonomy, its benefits and the limitations?

CAV: Molecular taxonomy is most important as it provides an additional set of characters to be used in the classification of organisms. The taxonomy is all about judicious use and analysis of inheritable characters to arrive at a classification hypothesis. Characters may be behavioral, chemical, morphological and molecular. The species concepts and higher classification (subtribes, tribes, subfamilies, families, etc.) will be improved by making them monophyletic based on molecular characters. In addition, one will be able to unravel the problems of sibling species, which are of utmost importance in vector species of human and plant diseases and in search of proper biological control agents of pest species especially the invasive organisms.

More importantly we can relate different stages of insects with molecular taxonomy where early detection of the occurrence of the pest helps in developing a suitable pest management strategies. It's of utmost importance in quarantine too, where most of the invasive species enter into new territories in cryptic form (in egg and larval stages), which can be easily detected by molecular tools. It helps in redefining species concepts. But there is a problem of estimation of extent of homoplasy even in the molecular characters. In conventional taxonomy, the homologous, non-homoplasious characters are considered for phylogenetic analysis and hence the technologies have to be developed even in molecular characters to address the problem of homoplasies.

KS: In India, most taxonomists are carrying their studies at alpha level, in this context what are the ways and means to improve or take taxonomic studies to next level?

CAV: As I have emphasized earlier, the collections are more important. Lack of collections from various zoo-geographical regions of the world in addition to emphasis on the study of local fauna (we know probably only about 30% of our fauna) are the reasons for most of our taxonomic work being at alpha level. For Beta level taxonomy, representation of major taxa from most zoogeogrophical regions of the world is required. Even our National Institutes like Zoological Survey of India (ZSI), Forest Research Institute (FRI), Indian Agricultural Research Institute (IARI) do not have taxa representing different zoogeographical regions of the world. Most of these museums also do not

have good representation of taxa described form the Indian region also. It is very important to have an Institute that can house most of the taxa if not all, known arthropods from India with facilities for curation and preservation of international standards and provide an authentic identification service for persons who need such a help. It should also be accessible for all students (both national and international) of Arthropods to utilize these specimens for their research work.

KS: May we know the other activities off the taxonomic research and teaching that relaxes you?

CAV: I relax only when I work on leafhoppers especially some groups like Idiocerinae and Opsiini as leafhopper taxonomy is my passion. When I am back at home, I watch TV and would like to watch mostly detective movies. I like James Bond movies, Sherlock homes series, etc. I feel that the taxonomic research is also like detective work only, to find out it's origin, dig out the literature and find the location of types and come out with new outcomes, new findings, new discoveries….and nodded his head with a sweet smile on face indicating 'that's it'

Concluding remarks by KS:

I was spellbound by the passion infused in every word of the legendary taxonomist, Dr. Chandrashekharaswami A. Viraktamath. **Even at the age nearing 79, Dr. CAV is energetic to work with the minute leafhoppers and other insects and expressed the happiness in catering identification services and guiding students and researchers. Dr. CAV expressed that his post retirement work on leafhopper taxonomy was more voluminous than that was accomplished during 35 years of active service. This speaks his untiring efforts, commitment, dedication and contribution towards insect taxonomy.** It was a sheer delight and privilege interviewing legendary CAV Sir, which was an enlightening experience listening to his rich and decades of vast and varied experiences interspersed with interesting anecdotes.

The interview was conducted by Dr. Kolla Sreedevi. She is working as Pr. Scientist at Division of Germplasm Collection and Characterisation, ICAR- NBAIR, Bengaluru. She is working in the field of Insect Biodiversity and Systematics especially Coleoptera; Insect Ecology, biogeography and molecular characteri-sation. She is also an Associate Editor of IE.

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Callerebia hybrida Butler, 1880 – (Lepidoptera; Nymphalidae) a new addition to the butterflies of union territory of Jammu and Kashmir, India

Nazim Ali Khan and Taslima Sheikh

From Varshney & Smetacek (2015), this butterfly has range extension from Himachal Pradesh to Uttarakhand. According to Gasse (2018), this is often seen in Himalayas from 900 to 3900m, in west Kangra (Himachal Pradesh) and eastern Uttarakhand upto central Nepal and has been recorded from Bhutan also. The species is written as *Erebia hybrida* (Hybrid Argus) in Evans, 1932, and in Talbot, 1947. Dachal area lies in the foothills of Pir Panjal range within the Himalayas. The highest summer temperature is in between 19-35℃.

Material and Methods:

On 05-vi-2021, first author N.A.K has done survey on the butterflies of Dachal area in Mendhar city (Fig. 1) in district Poonch of Jammu and Kashmir Union Territory where he observed *Callerebia hybrida* butterfly (Fig. 2) puddling on soil. He photographed this butterfly with the help of Canon EOS 200D II, and noted down the coordinates of that area (33°36’02’’ N, 74°08’52’’ E) at an altitude of around 1580m. This butterfly individual was further identified with the help of following literatures: Evans (1932), Talbot (1947), Wynter-Blyth (1957), Kehimkar (2016) Smetacek (2018). We also checked the recent publications on butterfly diversity of Jammu and Kashmir Himalaya i.e., Sheikh & Parey 2019a, 2019b; Gupta & Sheikh, 2021; Sheikh, 2021; Singh & Sheikh, 2021 and Parey & Sheikh, 2021.

This butterfly was not seen or observed earlier from Jammu and Kashmir Union Territory, India (Sheikh *et al.* 2021) and thus occurrence of this butterfly species can be stated as new report from the Union Territory of Jammu and Kashmir. After this find of butterfly, Jammu and Kashmir Butterflies checklist has reached its number to 311.

Taxonomy:

Order: Lepidoptera

Superfamily: Papilionoidea

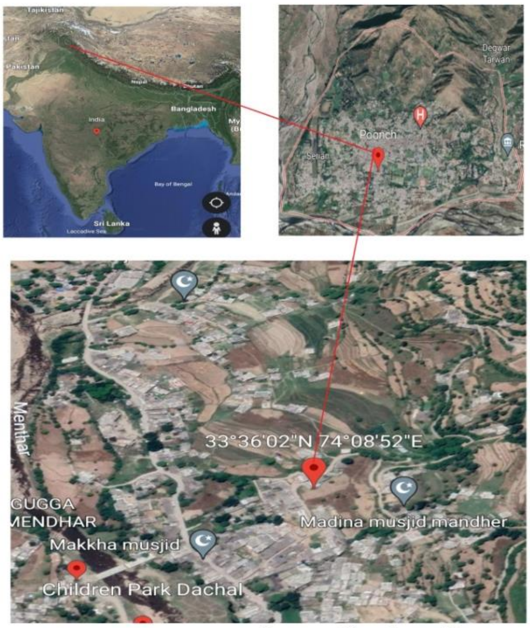
Family: Nymphalidae

Subfamily: Satyrinae

Features: The species can be identified by observing two eyespots on underside hindwing and a series of white discal spots above. In the central area and sub marginal area brown band or dark line can be seen. Marginal dark line of underside hindwing does not touch the eyespots. Forewing apex rounded.

Conclusion:

This addition of species has increased the butterfly checklist number to 311. More observation of this species can tell us the diversity status of this butterfly in UT of J&K. More studies should be carried out to find its host plant as well as nectar plants.



Source: Google earth maps

Fig 1: Map showing *Callerebia hybrida* location

Fig 2: *Callerebia hybrida* Butler

Acknowledgement:

Authors want to thank Mr. Lovish Garlani for the confirmation about the identification of the species.

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Charismatic butterflies with bizarre etiquette: "Necrophagy and Kleptopharmacophagy"

Deeksha M G, Mritunjoy Barman and Himanshu Thakur

Butterflies are the exquisite diurnal creatures that makes nature so spectacular. Scientists and amateurs across the globe have been fascinated by their radiant colour patterns, ephemeral appearance, confounding life history, and immense diversity. Butterflies are the short-lived adult stage of the insect order 'Lepidoptera', which are spotted fluttering by plants to feed nectar from flowers and/or to lay eggs in 'host plants', where the caterpillar would be feeding

Adult butterflies exhibit some curious behaviour *viz*., basking, perching, patrolling, and puddling, these are unexplored knowledge to many common people. Basking is a warming up process of butterflies' wing muscles to take flight commonly observed in Nymphalidae and Pieridae family butterflies, as they are cold blooded organism and require around 24°C to 32°C to take flight, so this thermoregulation process help to initiate flight activity in butterflies (Akand *et al*., 2018). Actions like patrolling and perching are adopted by the male butterfly during procreating, where they search the female butterfly by these actions. In case of patrolling male butterfly take a flight in the place where they are visiting flower for nectar collection or searching site for egg laying, once it finds the female of same species in began its courtship, this is commonly observed in Monarchs and Sulphurs. But in some butterfly like Black Swallowtail and Red Admiral males perch in the tall plants near the water source where the probability of female visitation is high, once it locates the female of same species the courting ritual starts (Krischik, 1996). Whereas, puddling is mainly performed by a male butterfly to procure the minerals from moist soil, this helps butterfly to mate successfully as male pass the obtained nutrients through sperm, it assists female in reproduction. Sometimes it aids in pheromone production by male to attract female (Cannon, 2019).

Other than this butterfly shows some acts like feeding on a dead organism, this behavior is known as "necrophagy" mainly to obtain some mineral salts and amino acids. This behavior mainly occurs when the nectar of the flower is unable to meet the nutrient requirement of the adult butterfly they find an alternative source like a dead organism, to accomplish their requirements for reproduction (Payne and King 1969; Gu *et al*., 2014). In this article, the necrophage behaviour of butterfly observed in the place Madikeri of Kodagu district in Karnataka (12°24'04″ N, 75°43'36.1″ E) on 22 May 2021 is presented in Plate 1. Where *Mycalesis junonia* Butler (Malabar Glad-eye Bushbrown butterfly) of Nymphalidae family is spotted siphoning on the carcass of grasshopper. Though it's a surprising proclivity of butterflies, they have developed this behaviour to derive the nutrients to complete the adult stage systematically.

Recently some neoteric studies on the "kleptopharmacophagy"- a neologism explaining the chemical theft between living organisms, this nature of the milkweed butterfly are gaining the attention of the biologist. The milkweed butterflies have a chemical thieving behavior, where they attack the caterpillar of own species to obtain the defensive chemicals from wounded caterpillars which feeds on the milkweed to get the phytochemical defense against its enemy, but the adult sucks the internal liquid of the caterpillar by wounding them to acquire the toxic alkaloids, thus inbuild their defence system, also these alkaloids act as a biochemical precursor of their mating pheromone synthesis. If same process is observed under the condition, where adults feed on dead caterpillar it is known as “necropharmacophagy” (Tea et al., 2021). Though, this recent study has many queries to be cleared, but has given foundational information to the biologist to work more, in order to decipher the accurate reason behind this behaviour in butterflies.

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Plate 1. *Mycalesis junonia* feeding on the grasshopper cadaver

A tarsonemid mite feeding on uredospores of linseed rust in West Bengal, India

Priyankar Mondal, Arka Manna, Sk. Hafijur Rahaman, Lakshman Ch. Patel and Krishna Karmakar

The study of plant inhabiting mites (Acari) in India has increased in the last two decades due to growing recognition of their importance both as pestiferous species and natural enemies (Gupta, 1985; Singh and Raghuraman, 2011; Gupta & Karmakar, 2015). However, there is still a general lack of knowledge of the biology, reproduction, behaviour, ecological associations, symptomatology and taxonomy of these mites. The family Tarsonemidae (Acari: Heterostigmata) consists of 45 genera and more than 600 species worldwide but only a handful of species have been reported from India (Mondal *et al*., 2021; Ganguly *et al*., 2021). Tarsonemid mites are very tiny (90-300 µm in length) with shiny, translucent oval-shaped bodies, sometimes having yellowish to dark brown tinges, generally inhabiting cryptic microniches, preferably the humid areas. They play a diverse role in terrestrial ecosystems by feeding on plants, epiphytotic microbes, predating on mite eggs, parasitizing insects, using insects for phoresy and also functioning as parasitoids of insects (Lindquist, 1986; Mondal & Karmakar, 2021). Among the plant-associated tarsonemid mites, two species viz. *Polyphagotarsonemus latus* (broad mite/yellow mite) and *Steneotarsonemus spinki* (sheath mite) have drawn lots of attention due to their yield-reducing potential and havoc infestation on several agri-horticultural crops and rice respectively while the rest of the species remains unnoticed (Karmakar, 2008; Gupta, 2012).

Very recently in March, 2022 we have observed a fairly large population of *Tarsonemus mondouriensis* Karmakar & Ganguly, 2021 feeding on uredospores of *Melamspora lini* (Fungi: Basidiomycotina) on leaves of linseed (*Linum usitattisimum* L.) and also the necrotic parts of infected leaves (Figure 1). The fungus *M. lini* is the causal organism of the linseed rust disease which appear late in the season drastically reducing seed yield, oil content and fibre quality (Singh *et al*., 2017). Historically, *M. lini* is the 'famous' plant pathogen that led Harold Flor to postulate the "gene-for-gene" hypothesis in 1942 which revolutionized inheritance studies in host-parasite interaction systems. Till now, no mite species has been reported to be feeding on or associated with the uredospores of *M. lini*. However, it is not surprising to find *T. mondouriensis* feeding on its spores as many of the *Tarsonemus* species have already been reported to feed on plant-parasitic fungal spores (mycophagous) worldwide (Lindquist, 1986; Lin & Zhang, 2002). Hofsetter and Moser (2014) showed that in some species of *Tarsonemus* the lateral enlargement of tergite C makes a flap-like structure ventrolaterally which serves as a pocket to store and transport fungal spores, often termed as 'sporotheca'. A similar structure can also be observed in the original illustration of this species by Mondal *et al.* (2021) although it is not described as sporotheca. However, their statement regarding locating this species in fungus infested rice grains indicated towards a trophic association with plant pathogenic fungus.

We further observed that males were very low in numbers (only 2) compared to females (23 approx.) in 80 infested leaves sampled during this study demonstrating a female-biased sex ratio that commemorates with the sex ratios (from 2.3:1 to 8.4: 1) found in other *Tarsonemus* and *Steneotarsonemus* spp. by Karl (1965) and White & Sinha (1981). During our observation, some female mites were found depositing eggs inside the pustules surrounded by uredospores but the exact cause could not be determined (see supplementary video uploaded on website).

We hope this field note will help the plant protection workers to look for more tarsonemid mites occurring in the prevailing agroecosystems of our region and understand their relationship with other organisms.

Author's contribution:

AM, SHR & LCP observed the mites on rust infected linseed leaves for the first time. AM & SHR took population count. PM & KK investigated further both in field and laboratory, identified the mite specimens and prepared the manuscript.

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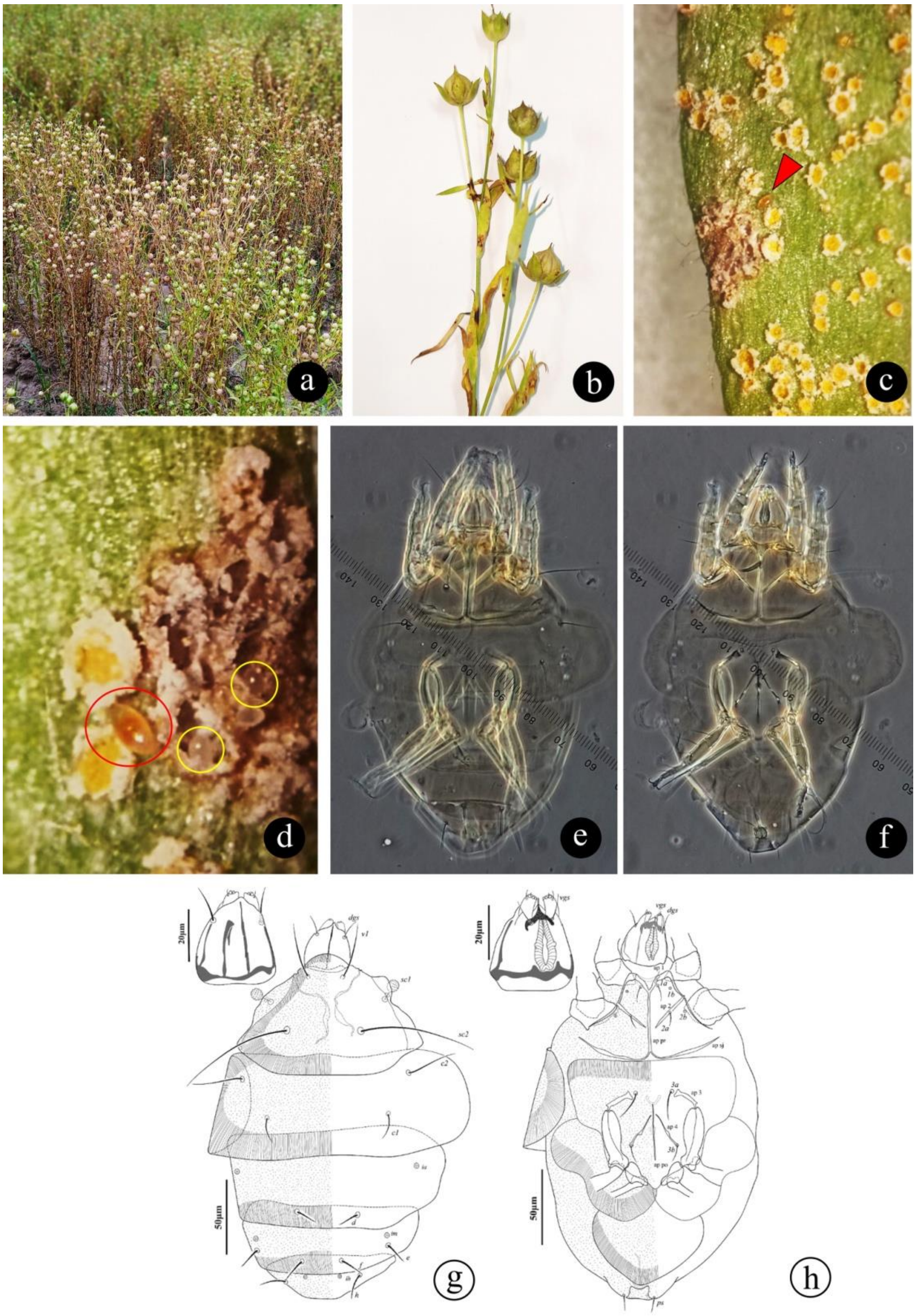

Fig. 1: a) Linseed field infested with *Melamspora lini*; b) rust infected linseed plant; c) uredopustules on leaf, red triangle indicating the female mite; d) a small colony of mites; red circle indicating adult female and yellow circles indicating translucent eggs; *T. mondouriensis* adult female phase micrograph dorsal (e) and ventral (f) (40X zoom, Olympus BX53); *T. mondouriensis* adult female vector illustration dorsal (g) and ventral (h), adapted from Mondal *et al*., 2021

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First record of *Euchariomyia dives* Bigot,1888 (Diptera, Bombyliidae) from Jessore Sloth Bear Sanctury, Gujarat, India

Anuj D Raina, Kailash Rameshwar Jani and Akshay Chauhan

Abstract: *Euchariomyia dives*, Bigot 1888 belonging to the family Bombyliidae is recorded from Jessore Sloth Bear Sanctuary in Gujarat State, India for the first time. The species has not been reported from Gujarat or any other dry-deciduous regions of India earlier.

Key words: *Euchariomyia*, Bombyliinae, *Euchariomyia dives*, dry-deciduous forest, Jessore Sloth Bear Sanctuary.

The subfamily Bombyliinae constitutes 72 genus comprising 1155 species (Catalogue of Life, 2021). India is home to 138 species assigned under 36 genera, 11 tribes and 8 subfamilies (Banerjee and Mitra, 2006). Bombyliinae is one of the largest subfamilies of bee flies with diversity higher in southern hemisphere than in the northern hemisphere (Li and Yeates, 2019). The parasitoid larvae of Bombyliinae feeds on immature stages of Orthoptera, Coleoptera and Hymenoptera (Yeates and Greathead, 1997). Bombyliinae subfamily includes a monotypic genus Euchariomyia (Hull, 1973). The genus exhibits sexual dimorphism with high variation in coloration of same sex specimens (Evenhuis and Gang, 2016). *Euchariomyia dives* is only species listed under *Euchariomyia* genus making it monotypic (COL, 2021).

Results and Discussion:

Systematics
Phylum: Arthropoda
Class: Insecta
Order: Diptera
Family: Bombyliidae
Subfamily: Bombyliinae
Tribe: Bombyliini
Genus: *Euchariomyia*
Species: *Euchariomyia dives* Bigot, 1888

Diagnosis:

Based on the description by Evenhuis and Gang, 2016 we have listed a few identical morphological features that are readily noticeable in the photographs captured. Head: black with sparse hairs, male eyes are holoptic whereas female eyes are dichoptic. Presence of white scales above antennae on both sexes. Proboscis: black in both male and female with size three times the head. Wing: infuscated or dark brown, alula and lobe present with base of brown hair on edges. Thorax: scutellum brown pollinose; hairs and tomentum on thorax mostly orange yellow, postpronotal lobe with orange yellow long hairs, mesonotum with orange yellow long hairs anteriorly; scutellum is metallic blue shiny and almost bare, with orange yellow tomentum and opalescent scales anteriorly and laterally; scutellum with black bristles and thinner black hairs on posterior edge. Abdomen: black with hairs; female dorsum with dense fiery red or orange recumbent tomentum and few erect black

Fig 1. Mating behavior of *Euchariomyia dives* at Jessore Sloth Bear Sanctury. Photographs by Anuj D. Raina.

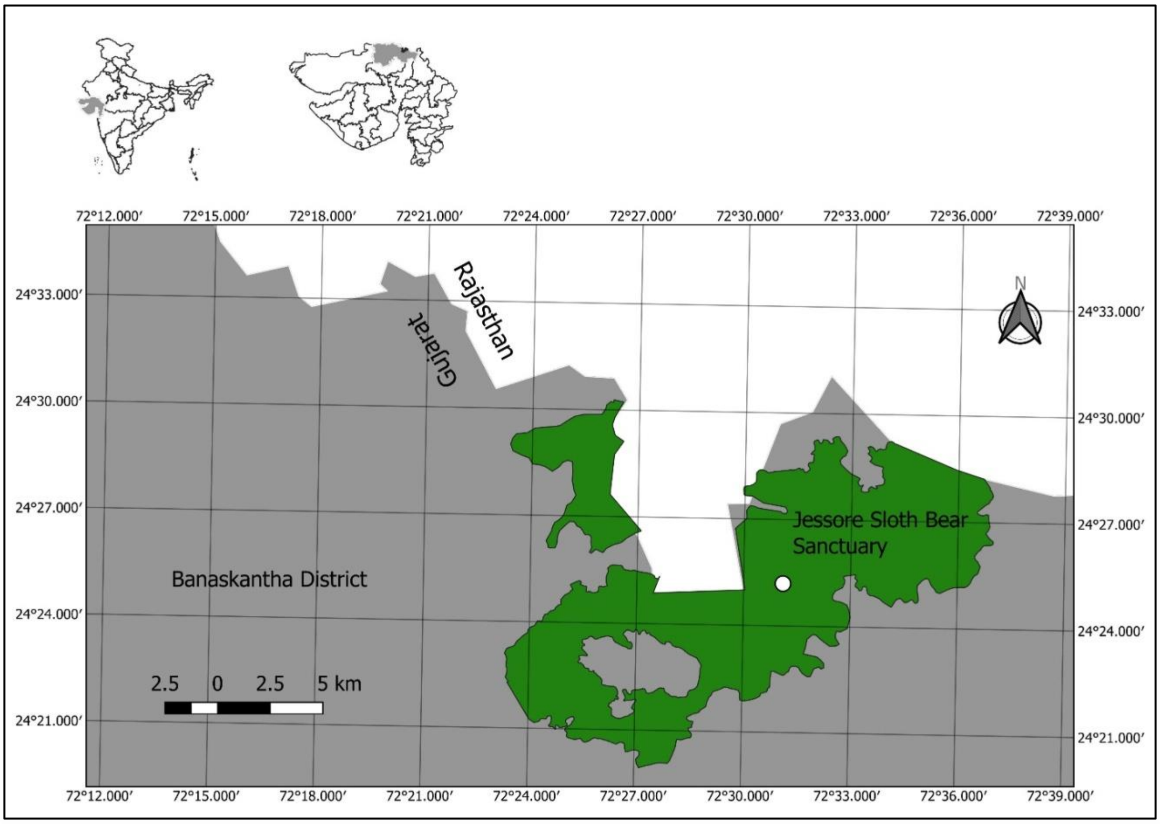

Fig 2. Map showing Jessore Sloth Bear Sanctuary on Gujarat-Rajasthan border and white point of *Euchariomyia dives* sighting.

hairs on most tergites, whereas male has black abdomen having white silvery dense scales. Both sexes having sternites with black hairs and broad oval abdomen. Legs: long slender, dark brown; coxae with all hairs black, setae on legs black; femora with long sparse black hairs; tibiae and tarsi with short black hairs.

Distribution:

Yao *et al*. (2009), describes its distribution in oriental regions of India (Kerala, Orissa, Tamil Nadu, Uttar Pradesh), Indonesia (Java), Laos, Sri Lanka, Thailand and Paleartic Region of China. Dhamorikar 2017 has listed the species from Mumbai Metropolitan Region, Maharashtra. So far, no published record from Gujarat exists.

Remarks:

Euchariomyia dives, Bigot 1888 was sighted mating in Jessore Sloth Bear Sanctuary for first time on 24th June 2017 6:50 PM IST (coordinate 24.419862, 72.518392; altitude 645m asl).

During trekking and passing through grass vegetation, the *Euchariomyia dives* duo got disturbed and took slow swift flight shifting to safe point. The species was well photographed from all directions to note morphological features. The identification of photographs and characters were done using available literature (Yao *et al*., 2009).

The forest has witnessed early shower, while the maximum rainfall of monsoon occurs during the month of July-August. The protected area of Sanctuary has hilly terrain covered with rocks. The Sanctuary lies in western region of Aravali range sharing close proximity with Mt Abu Wildlife Sanctuary of Rajasthan (Mt Abu: Highest peak of Aravali) and Balaram Ambaji Wildlife Sanctuary of Gujarat. The dry deciduous forest type of ecosystem consists of arid to semi-arid and dry deciduous thorny scrub vegetation. The aerial distance as shown in Google map for *Euchariomyia dives* sighting and Rajasthan state is 1.06 km (approximate).

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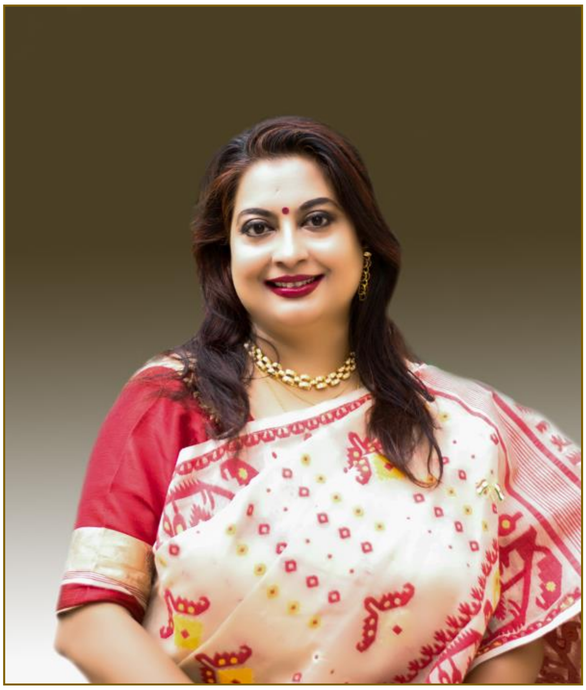
In Conversation with India's first woman chief of ZSI in 100 years: Dr. Dhriti Banerjee

A woman with illustrious scientific career, an entomologist with expertise in order Diptera worked on taxonomy, zoogeography, morphology and molecular systematics shares her journey in ZSI with IE associate editor Dr. Bhagyashree.

Dr. Dhriti Banerjee, the first woman Director of Zoological Survey of India, in the 106-year history of ZSI, a premier research institution on Faunal Biodiversity and Conservation under the aegis of the Ministry of Environment Forests and Climate Change, Government of India. She was born and brought up with school and college education in Kolkata. She did her under graduation and post graduation in Zoology from Calcutta University and Ph. D from Kalyani University, Nadia. She began her research career on the physiological effects of sustained use of abusive drugs in human and animals and was awarded with several fellowships and grants for her research projects. On completion of her Ph. D., she joined the Zoological Survey of India in 1998 as a junior research scientist, with in stint of two decades she has assumed the charge of Director. She is an entomologist with specialisation on flies (Diptera), her research interest includes taxonomy, molecular systematics, biodiversity, distribution, GIS mapping and climate modelling of flies and also on forensic dipterology. She had initiated molecular research in ZSI way back in 2011. Known as the Lady of the Flies she is also



into the science of Criminal Forensics and has found viable models of fly species which can be used for crime detection in Indian environmental conditions to aid law enforcing agencies. She was the part of the detective team which traced the grave of ZSI's first director-general Thomas Nelson Annandale, who was buried at the Scottish Cemetery in April 1924. She has spearheaded the ZSI faunal information system, multi-dimensional platform housing information, collections, spatial and temporal data as well as genetics and molecular information about the faunal species.

Dr. Dhriti Banerjee is the lady behind the Digital ZSI; she was instrumental in digital ZSI initiatives as a co-ordinator of ZSI's Digital Sequence Information Project under which unique databases were established *viz*., Fauna of India digital library, ZSIFIS (Zoological Survey of India Faunal Information System) and ZSI Mobile APP for Protected Areas. This web-based information would enable all researchers, wildlife enthusiasts, students, policy managers, conservationists and anyone interested in biodiversity to access the specimens as well as available information of the National Zoological Collection and the Fauna of India by the click of a mouse. She has authored 26 books, 49 book chapters and 219 research articles in peer reviewed repute national and international journals and a few popular articles in vernacular language. Dr. Dhriti Banerjee is well decorated with awards and honours for her dedication towards faunistic studies of Diptera. She was conferred with the award of the "Exceptional Woman of Excellence", by the Women's Economic Forum, at The Hague, Netherlands on the International Women's Day, 2018. Very recently she has been awarded the "Amazing Godavari Memorial Award for Excellence in the field of Education and Science" on the 30th January 2022. Dr. Banerjee has also been awarded with Nari Shakti Samman Purashkar, 2022 by Society of Biological Sciences and Rural Development, Prayagraj in the celebration of International Women's Day, 2022. She has also been awarded with H R Mehra award by The Helminthological Society of India on 21st March, 2022.

> **"There is no glass ceiling to break. It's only the chain – mental and emotional – that the women need to get rid of"**

Dr. Bhagyashree S N (BSN): Firstly, congratulations on being first women Director of the mega organization, tell me, how was your feeling after breaking glass ceiling at ZSI by becoming the India's first women director of ZSI?

Dr. Dhriti Banerjee (DB): Thank you very much. But frankly, I don't make much of this glass ceiling business. It is a much-hyped statement, only to make women feel a little better about themselves. Women have always demonstrated enough grit to not just lead but lead with admirable poise and grace. Draupadi, Cleopatra, Nefertiti, Razia Sultan, Madam Curie, Queens Victoria and Elizabeth, Golda Meir, Srimavo Bandaranayake, Indira Gandhi, Indra Nooyi are names that stand and tell you that women reaching the top is not so much of an exception. In fact, that should be the norm. The trouble is that the male dominated societies globally have kept women tied down to home and hearth. That's the reason why you see fewer than desirable number of women at the top. There is no glass ceiling to break. It's only the chain – mental and emotional – that the women need to get rid of. Of course, it feels good to reach here and I sincerely hope it will work as an inspiration for many more women to break the shackles.

BSN: What was your childhood like and what inspired you to be the women you are today?

DB: It was an ordinary childhood. As an only daughter, I was pampered but in a large extended family with number of brothers

This is how you feel when you've reached the TOP!

and sisters, comparisons were natural. I always felt a little less than others. I was blessed in that my father, like most Bengali fathers, made me feel special. For him, I was the brightest and sharpest child on earth. His expectations, coupled with a tough disciplinarian mother made me work harder at everything that I was supposed to do. I learnt to define my standards early in life and that has stuck. Getting inspired was not important. Getting it right (by my standards) was. I was always scared of failing to meet my father's expectations and my mother's standards. That helped me excel as a student. As I grew, I could see that women were spoken of more in terms of their physical appearance and beauty rather than their intellectual capabilities and accomplishments. I found it hard to accept. As I read more and more about women who were achievers, I developed this strong need to be identified more for my achievements and accomplishments rather than for my physical appearance. That perhaps was my biggest motivator and inspiration.

BSN: How did you choose to become an entomologist especially taxonomist, is it by choice or by chance?

DB: I didn't exactly choose to be an Entomologist. I was trained in physiology, Bio-chemistry and Hematology. I was selected simultaneously for the post of Lecturer in Zoology at a State College and for ZSI. The appointment letter for ZSI came before that of the Lecturer post with a joining window of 1 month. So, I joined ZSI first and I guess it was destiny's choice that I join ZSI.

Figuring out if it's a fly of interest

BSN: How was your journey at ZSI? Among so many Insect orders, why did you choose Dipterans??

DB: When I joined ZSI, nobody was sure what and where I should be working. In the first one month, I didn't even have a place to sit. After nearly a month there were two departments that needed someone. I was not given any option and I was posted in the Diptera section. While at the university I was always fascinated by how you could calculate the time of a death or murder simply by examining the flies around the dead body. So, when I was given the choice, it was quite natural that I would opt for it. However, in hindsight, it was quite a good choice. There is so much to explore and learn about two winged flies and insects, it's amazing. It's like an entirely different universe. I am still fascinated by the element of surprise in our new findings and discoveries of new species which ZSI is doing in a regular basis.

BSN: Usually it's human tendency to feel like, if I become Director, I would change this, I would do this, I would make this happen or doing something different, before becoming director what were the

changes you dreamt of making and what were your imaginary visions?

DB: You must remember, before becoming the Director, I was Head of Office for a good 5 years from 2010 to 2015. Previous to that I was the Drawing & Disbursing Officer of ZSI. I was also Chairman of several committees including the Canteen Committee and also the General Secretary of the Recreation Club of ZSI. I have been working here since I was 28 years old. After 25 years I realize that there is no aspect of the organization that is unknown to me.

"You cannot evade my net"

From administration and scientific work to staff welfare association and recreational activities, there is no pie in which I didn't have my hand in. Nothing in ZSI was new to me when I assumed the responsibility of the Director, ZSI. Administration and issues related to administration are not new to me. That exposure has also allowed me to remain grounded and understand that you cannot – in fact you should not – seek drastic changes. No matter how flawed a system or a process may appear, there is always some merit and logic in it. I was clear in my head that I do not need to dismantle the system. Yes, there is a need for a few changes, but those changes need to be brought in without any major upheaval. That is at the system and process level. The one area that I always wanted to work upon was the culture. We publish a lot of research paper. We have some of the finest scientists and researchers. But most of our work has been done in isolation. There hasn't been much collaboration or coordination with other agencies in India that are working in related fields, nor have we made much of a headway in terms of reaching out to scientists in other parts of world. I hope to build a culture of collaboration that would allow us set international benchmarks.

Leading the first all women high altitude expedition of ZSI

Dr. Dhriti Banarjee at her office

BSN: Do you think biological clock and family pressure makes a women to take a back steps from aiming high/reaching greater heights in her career, though men and women are cerebrally equal in scientific and administrative skills?

DB: I have a slightly different view. Most women are sharper than men. It's not a hollow claim. I was the top student in my class and women were in a minority in my class. Look at the 3 IAS toppers this year/ If you look at percentage representation of women, that is an amazing success rate. I believe women are sharper and more focused. The reason only a limited number makes it to the top, has more to do with the social norms and taboos than with the biological clock – as you put it. Apart from nursing a child, I don't see why a man cannot be an equal partner in raising children or taking care of the elderly in the family. That is all that needs to be done. Along with maternity leave allow for paternity leave as well so that a husband can reduce the burden of a young mother and she can get back to work early enough after child birth. Stop expecting a woman to look after the elderly all by herself. The man must support her. If the responsibilities are equally shared, you will have the whole dynamics changing. You will not need to worry about the biological clock then.

"I believe that a woman has the power to take an entire world."

BSN: How would you like to see "Indian Taxonomy" in future?

DB: Indian Taxonomy needs to have more experts covering all lesser-known groups. The subject needs to reinvent itself to draw more young blood. There needs to be more job opportunity than we have today. The taxonomy should have a wider perspective and should be put into much wider use. Only then the popularity of the subject and the importance of taxonomy will be in the rise.

Identifying species will be the mainstay of all biological research and all biologically used organisms mandatorily should be given a name but not be denoted by a number. For example, not every gene sequence uploaded in the NCBI has a name and it's designated by number. This easy designation of the species is an impediment to taxonomic expertise. There should be a diktat in all research related to basic biological sciences, criminal and forensic sciences and biotechnology that all biological species involved should be assigned a name. Through this process the education system and the industry would be forced to give recognition to taxonomists and there would be mandatory positions in these sectors for taxonomists to get gainful employment.

BSN: So many taxonomists are getting retired and this is the one of the major impediments in the field of taxonomy? What are you plans solve this??

DB: The problem can be solved only if the retired scientists are hired as consultants in the different institutes. They should also be assigned the responsibility of not only filling the gaps in research but also aim at grooming the second line of expertise in a particular field or taxa.

BSN: Many young taxonomists are just doing contract job and switching to other areas? And they are not confident in job stability in taxonomy due to the dearth of opportunities, what are you plans to create opportunities for young ambitious and desirable taxonomists?

DB: At present ZSI is working with fifty percent of its staff strength. The MoEFCC has plans for filling up all the vacancies in a mission mode. So, I am hopeful that Zoological Survey of India will be able to provide opportunities for young ambitious and desirable taxonomists in the years to come. However, young and ambitious have to keep their eyes and ears open for the posts to be advertised and apply according to their expertise.

BSN: Do you think women feel like "An ants in the elephant world" when it comes to taking mega administrative job? And what are your suggestions to such desirable women's who are giving up?

DB: I don't think so. In fact, women are conditioned to believe that "sacrificing" her interest for the greater good of the family is a noble act and therefore she must do it. And she does it gladly. Only to realize later in life that it wasn't worth it. I do not think women feel threatened or trampled upon. It's just that they are made to believe that being a "Sita" is her best choice. I worship Ma Durga. I believe that a woman has the power to take an entire world. It's just that she needs to know what she is capable. That's all!

A groupie with the most resilient Women of ZSI

BSN: When I was a student of taxonomy, major problem I have faced is inaccessibility of Indian types for study and comparisons while describing new species and to do revisions, how to overcome this?

DB: Inaccessibility of Type-Specimens for research may be addressed by visiting the institutions in India where the Type-Specimen are available. Any field of research whether applied or theoretical will have its own problems and all researchers should be capable of addressing the problem accordingly. Several Type-Specimen are available in digital platform and now a days it's a lot easier to access the Types than when I started my work in this field. Moreover, most museums and taxa experts readily share images with scientists in India since we are not allowed to send any biological material abroad. Accessing Types is an impediment but not an insurmountable one.

BSN: In general, Taxonomy is male dominated world as it involves loads of field expedition, when it comes to other ecosystem like aquatic, forest and wildlife fauna, it's really challenging for women, how can a women cope up with this?

DB: Gregor Mendel was not a gym goer neither was Rosalind Franklin. Like I have mentioned a little while ago, it's all in the head. I have been in the field and I have worked shoulder to shoulder with the men and I can say, no less effectively. Yes, there are a few challenges. For example, access to a decent hygienic toilette. But then, the men have struggles of different kind as well. It's up to a woman to decide how she is going to cope up with the challenges. If it's not this then it would be something else. One needs to be clear about what she wants to achieve. That would make every challenge seem like routine and not too difficult.

BSN: When faced with obstacles or hardships or challenges, where do you find the strength to overcome it?

DB: I worship Ma Durga. The way she took on an entire army of Asuras is inspiration enough. Every time I find myself in a challenging situation, I think of the challenge as an Asura to be slain. By the way Dhriti is another name of Ma Durga!

BSN: Can you tell me one female mentor or an inspiring female figure in your life?

DB: My mother. I know there are enough women in this world to be hero-worshipped. But if there is one woman who has been an inspiration it's my mother. I also like to add here my daughter happens to be my biggest cheer leader. I was always supported by my in-laws and my husband all through. I was never questioned on my decisions. The onus of it always rested on me. So, I guess I didn't have anybody to blame when anything went wrong. This was something I had learnt very early from my father. I could take decisions in most cases without consultations. That helped in a big way in making me own the laurels if they went right and also the responsibility of rectifying them solely rested on me when it went wrong.

BSN: My last question! As you are expert in digitalization, what are your visions on "Digitalization of Indian Taxonomy"?

DB: Digitalization of Taxonomic specimens is now the call of the day. It helps the custodian of the specimens in preventing rough handling and damage to the priceless specimens. At the same time, it helps the students of Taxonomy in accessing the maximum number of specimens at the click of a mouse. In today's tech-savy world that the accessibility of specimen images to one and all automatically triggers enthusiasm in both students of Biology and students of

Mathematics, Computer and Engineering. Availability of photographs in your mobile phone, Instagram and Facebook will allow a person sitting in a remote village to know about the local fauna in his or her area. The future of Indian Taxonomy will be like "Har Ghar mein Taxonomists!".

"You may say I'm a dreamer

But I'm not the only one.

I hope someday you'll join us,

And the world will be as one."

……John Winston Lenon.

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Insect ectoparasites: A driving force in the evolution of zebra stripes

K Chandrakumara, Arunkumara C G, Mukesh K Dhillon, Vinay K Kalia and K Srinivas

Abstract: Zebras are well-known for their distinctive striping pattern. The presence of contrastive white and black stripes has been attributed to camouflage, predator avoidance, thermoregulation, social interaction, and ectoparasite avoidance. According to latest studies, the stripes on zebras are essentially evolved to ward off biting flies. The presence of alternate black and white stripes causes the disruption of optic flow patterns, preventing the flies from landing. Flight trajectory studies also revealed the failure of landing by the biting flies particularly, tabanids. Evolutionary models have also confirmed the distribution of striped equids and biting flies. Striping characteristics such as thickness, number of stripes, and so on has also been treated to prevent biting flies. The genesis of zebra stripes has significance in animal husbandry as well.

Key words: Zebra stripes; Polarotactic tabanids; Evolution of stripes; Painted cows; African equids; Biting flies

Adopting any distinctive structure or behaviour in living creatures makes sense only when regarded as a series of evolutionary adaptations to specific selection pressures, implying that evolution is the cornerstone of biology and is fundamental to comprehending structural adaptations in the organisms. Likewise, zebras are several species of African equids united by their distinctive black-and-white striped coats. The unique stripes of zebras make them one of the animals most familiar to people. Their stripes come in different patterns, unique to each species, sometimes to subspecies also. As these contrasting black and white stripes are not found in other mammals, they are likely to have unique functions. Zebras have evolved among the Old-World horses within the last 4 million years (Azzaroli, 1992). It has been suggested that zebras are paraphyletic and that striped equids evolved more than once. However, molecular evidence supports zebras as a monophyletic lineage (Cirilli *et al.*, 2021). Zebras occur in an array of habitats, such as grasslands, savannah, woodlands, thorny scrublands, mountains, and coastal hills. The earliest report concerning the functional explanations for the stripes was started with Wallace who suggested that zebras have developed the striped coats as camouflage against carnivores in tall grass. Later, Darwin criticized his hypothesis with an explanation that zebras do not occur in such areas with dense vegetation but rather prefer open savannah habitats with short grass.

What could be the actual driving force for the evolution of stripes in zebras…?

The mysterious role of zebras' conspicuous black and white stripe pattern has been the focus of vigorous thoughts among researchers ever since Wallace and Darwin.

The meticulous functional explanations for these stripes were cloudy till the 19th century. Advanced studies related to the stripes function have given different explanations. There are 18 functional explanations suggested for the evolution of stripes in Zebras which can be folded into five main themes. These are camouflage, predator avoidance, heat management, social interaction, and avoiding ectoparasite attack (Horváth *et al*., 2018; Caro, 2020).

Camouflage is the eldest conception argued over by Wallace and Darwin. Early idea about the evolution of stripes was a form of crypsis/ camouflage to escape from the predators. Camouflage against predators may be expressed through background matching or else by disruptive colouration (Caro *et al.*, 2014). The recent evidence voices decisively contrary to this idea because a) Zebras are widely distributed in open plains of Africa (Savannah habitats) b) large carnivores can resolve stripes very easily and c) experiments showed that zebra stripes are quite noticeable to human observers (Caro *et al.*, 2020; How *et al.*, 2020). Hence, camouflage could not be a major driving force for the evolution of stripes. It sometimes refers to avoid being killed by predators through the confusion by misjudging the number of zebras in the group, the size of the target, flight speed, or through quality advertisement or aposematism (Caro *et al.*, 2014). Observations made on plains zebras which are escaping did not corroborate theories that stripes confuse predators in ways that do not enhance protean behaviour, nor obscure the outline of individual animals, and because they do not promote motion dazzle or cause lions to misdirect their attack (Caro, 2020). The worst part is that lions killed zebras in numerous numbers than expected based on their abundance in 40 study sites across Africa, implying that confusion is a doubtful operational explanation for stripes (Hayward and Kerley, 2005; How *et al.*, 2020). The other assumption was that stripes were meant to assist mutual grooming and thereby encourage social bonding. However, mutual grooming is not frequent anymore in striped than non-striped equid species. Given that unstriped domesticated horses (*Equus caballus*) clearly recognize each other, the argument that stripes are employed for individual recognition appears dubious. Furthermore, equid species that live in loose social groupings where individual difference is important do not have stripes (Caro *et al.*, 2014; Caro, 2020).

Zebra stripes are also thought to cool the body by forming convective air eddies across alternating black and white stripes due to the temperature disparities. According to the thermoregulation hypothesis, upwelling air streams originate over the warmer black stripes, which are then replaced by cooler air from the nearby white stripes with downwelling air flows (Horváth *et al*., 2018). Some glitches with this hypothesis are portrayed as follows: i) Hitherto, convective eddies formed over zebra bodies have never been recognized. Typically, these buoyancy-driven eddies form only on the horizontal regions of zebras' striped body surface. Upwelling turbulent airflow could build over the sharply sloped or vertical sections of the body, such as the flanks, avoiding periodic eddies. Black stripes, on the other hand, are detrimental to the slanting and vertical side areas, as well as the legs, because of their strong light absorption. It is vital to remember that zebras must be striped only on their dorsal surface, not all over their bodies, if they are to execute thermoregulation; ii) Convective eddies over horizontal surfaces are not well-known for their stability. The feeble local breeze, which is always present in bright conditions, might easily blow eddies away. Furthermore, these eddies could be quickly disrupted if the zebra moves (Caro *et al.*, 2014); iii) Herbivores sympatric with zebras that live in the same microhabitats also require thermoregulation yet they are not striped. Other equid species, such as horses, ass, *etc*., which live in hot climates, are also not striped (Caro *et al*., 2014).

Furthermore, it is unclear if convective eddies over a weeping horizontal surface of a striped coat have a greater cooling effect than sweat cooling over any monochromatic garment (Caro *et al.*, 2014; Caro, 2020). Additionally, the arena experiments which were conducted to investigate the functional explanation of stripes for thermoregulation by using water-filled metal barrels covered with horse, cattle and zebra hides of various colours such as black, white, grey and striped patterns revealed that there is no significant difference between the striped and grey barrels, even on many hot days. When the zebra-striped coats do not retain the body cooler than the grey coats, which implies that there is no correlation between thermoregulation and the presence of black and white stripes (Horváth *et al*., 2018).

Do stripes mean thwarting biting flies...?

Despite an adjoining perspective that zebras have thin pelage compared to other African ungulates, the equids outside of Africa have seasonally thick coats to combat the impact of low temperature (zebras which are distributed across Europe). Biting flies are very problematic for equids and they can pierce their mouthparts easily through the thin coat and skin of equids. The major parasites of zebras are tsetse flies, stable flies (*Stomoxys* sp), and tabanid biting flies, which are known to carry a range of diseases such as trypanosomiasis, equine infectious anaemia, African horse illness and equine influenza, which are fatal to zebras (Caro, 2020). The inkling that stripes oblige to thwart biting flies from landing started in the 1940s and has since been tested with various artificial targets independently in both field and laboratory settings. Among all postulates advanced so far why zebras have stripes, the avoidance of biting fly attack takes the most support (Caro *et al.*, 2014).

Mechanism of avoiding biting flies:

The evolutionary cause of zebra stripes is now widely accepted as avoiding attack by biting flies. However, the exact mechanism by which stripes protect against ectoparasite attack was unknown (Caro *et al.*, 2019). According to recent studies, stripes impede with the optic flow patterns necessary for tabanids to execute precise landings. Optic flow is the pattern of apparent motion caused by relative movement between an observer (Fly) and the scene (Zebra). The striped patterns, in particular, can disrupt optic flow by interfering with the radial symmetry of developing optic flow fields through the aperture effect of uniformly spaced stripes (i.e., production of false motion cues by straight edges) (How *et al.*, 2020). It is mainly due to the degree of polarization generated by black and white stripes. Black stripes exhibit highly polarized light with a high degree of polarization, whereas white stripes reflect light with a much lower degree of polarization diffusely backscatter light. White stripes disrupt the degree and angle of an otherwise attractive black pelage polarization signature. As tabanids and possibly glossinids can detect polarized light, but are unable to resolve the unpolarized light (Horváth *et al*., 2017; Egri *et al*., 2012; Britten *et al*., 2016; Caro *et al*., 2014; Caro, 2020).

Functional explanations for zebra stripes:

For nearly a century, the purpose of zebra stripes has never been thoroughly investigated. In multifactor models, Caro *et al*. (2014) compared accord discrepancy in the striping of equid species and subspecies to geographic range intersect of environmental variables. Association between the measure of striping and environmental factors (Camouflage, predator avoidance, thermal cooling and avoiding parasitic attack) was assessed at species and subspecies levels with different regions of interest (flank and rump striping, leg stripe intensity, facial and neck stripe number and shadow striping) by giving importance score to each environmental factor to highlight the prominence of stripes. At both species and subspecies levels for a different region of interest, avoiding tabanids (ectoparasites) received the highest importance score, which put forward those stripes have a role in thwarting biting flies.

However, phylogenetic analysis of equid subspecies, leg stripe intensity and tabanid activity indicated that the leg stripe intensity of equids is directly proportional to the avoidance of tabanid activity. It also highlights that those striped equids are better distributed in African countries than Europe. African equids have thin coat/ skin (due to temperature factor); hence they suffer the nuisance of tabanids. To overcome this menace, equids which are distributed across Africa evolved the white stripes (Caro *et al*., 2014).

Do stripe parameters influence the avoidance of biting flies?

The stripe parameters such as the number of stripes, horizontal and vertical stripes, the direction of polarization produced by the stripes and the thickness of the stripe may influence the ectoparasite activity. Egri *et al*. (2012) conducted various experiments at a Hungarian horse farm at Szokolya to investigate the influence of stripe parameters and polarization generated by them on the tabanid activity. The experiment concluded with the following results, i) the amount of tabanids captured was reduced as denser the white grid on white framed black plates filled with salad oil; ii) as the number of stripes increased, more the number of tabanids avoided; iii) as the thickness of white stripes reduced, greater the tabanids avoided; iv) the attractiveness of tabanids is influenced by the direction of polarization, regardless of the direction of striping and, v) stripes (horizontal or vertical) with the same brightness and colour but with alternating orthogonal directions of polarization are less preferred by tabanids than similar polarizing surfaces with a constant direction of polarization. The study with three-dimensional horse-shaped targets with different colour patterns (brown, black, white and a black-and-white zebra-striped) revealed a significant difference in the number of tabanids attracted to these horse models. Remarkably, fewer individuals are trapped with zebra-striped horse models than other horse models (Egri *et al*.,2012).

The behaviour of tabanids such as their landing, touching and hovering performance around the uniformly coloured domestic horses and captive plain zebras were examined in three different sets of studies such as direct observational, other coloured coatings and flight trajectory studies (Caro *et al.*, 2019). The experiment concluded that i) there was no significant difference in the frequency of circles and touches made by tabanids between zebras or horses. However, concerning landing, there was a significant difference between zebras and horses with more bias towards horses; ii) the touching and landing rates were tilted more towards monotonously coloured horses than the striped horses. In contrast, no significant differences were observed in the sum of flies that landed on horses' naked heads as they were not covered with any coat and iii) a significantly greater proportion of tabanids touched zebras as compared to horses. In contrast, a significantly lower proportion of tabanids landed on zebras than on horses.

The practical implications of the evolution:

Biting flies are solemn pests of livestock that cause economic losses in animal production. Kojima *et al.* (2019) anticipated that cows with black and white stripes on their bodies may avoid being bitten by flies and also displayed some fly-repelling behaviour. The experiment conducted on six Japanese Black cows assigned with different treatments such as black & white painted stripes (B&W), black painted stripes, and no stripes (all-black body surface) showed that the total number of biting flies on the legs, body, and the sum of both parts of B&W cows were almost equal to half of those number on the control- and black striped cows. The lower fly-repelling behaviour was observed in B&W cows which were convened by the fewer numbers of biting flies on their legs and body (Kojima *et al.*, 2019).

Zebra related interesting facts!!!

1. We might have observed a typical body painting pattern of different tribes living in Africa and Australia (Horváth *et al.*, 2019). Tribes generally dwell in the forests, where they are troubled by blood-sucking ectoparasites such as horseflies. Finally, we are perplexed as to whether tribes were aware of the scientific rationale for the practice of striping to avoid parasite assaults.

2. Further, other insects like the Colorado potato beetle, Hissing beetle (*Polyphylla decemlineata*), red cotton bug, *etc*., are also with different stripes on their body. Whether they also have the same evolutionary relationship to combat their natural enemies is unsolvable. However, in some insects like the red cotton bug, it is known that white stripes are formed by the accumulation of uric acid.

Conclusion:

Except for the ectoparasites, other possible functional hypotheses behind zebra stripes such as camouflage, predatory avoidance, decreasing thermal load, and social function, are still unconvinced and lack experimental data. Stripes with brightness and polarization modulations interrupting the homogenous pattern of reflected light may have evolved as a selective advantage in evading polarotactic biting flies. Apart from polarotaxis, more research is needed to uncover the possible processes of thwarting. Evolution is a two-way process in which both entities engage with one another to counteract each other's defense systems; however little information is linked to tabanid evolution concerning zebra striping. Finally, it is amazing to know that a larger animal, zebras, evolved solely because of a little fly parasite called tabanids.

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Bee decline: an ecological concern

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Abstract: The necessity for maintaining the ecological balance in equilibrium is a critical and pressing issue *vis-a-vis* the biodiversity of organisms including beneficial insects like honey bees and pollinators. Pollination is one of the greatest ecological services provided by insects with bees playing a significant role. Bee decline over the years has affected nature's equilibrium and has resulted in biodiversity loss, the consequence of which will be impaired food and nutritional security around the globe. The majority of the causes, which are man-made, may be checked if strict policies and guidelines are in place. Habitat loss, parasites and viruses, pesticides, monotonous diets, shipping fever, and climate change are some of the key causes leading to 'Bee decline'. Although not completely, concerted efforts toward an environmentally benign and conscious human activity could help to resurrect the vanishing bees and pollinators.

Key words: Bee decline, diseases, diversity, pollination, stressors

The diversity of wild bees and other pollinators has decreased over the last five decades, with a few species on the verge of extinction globally. In North America and Canada, as well as several European countries, managed honey bee numbers have collapsed (NRC, 2007). Apart from abiotic factors, almost 200,000 species of animal pollinators assist more than two-third of the world's angiosperms in meeting their reproductive needs to varying degrees. According to statistics, pollinator-plant interactions affect almost 4 lakh species. Pollination service is estimated to be worth 235-577 billion US dollars each year globally (Noriega *et al*., 2018). Pollination is a mutually beneficial interaction in which pollinating animals receive some form of nutritional 'reward' in exchange for transporting and visiting pollen. Pollen is a nutritive reward for some of the flies, butterflies, bees, and birds that do cross-pollination (Roulston *et al*., 2000).

Pollinator decline has reached such alarming levels that ecologists are warning that the world is on the cusp of a "pollination crisis," resulting in significant crop yield loss in the years ahead. Under future land use and climate change scenarios, an estimated five billion people could lose grain production due to insufficient pollination, notably in Africa and South Asia (Chaplin-Kramer *et al*., 2019). Reduced floral supply and nesting opportunities appear to be caused mostly by habitat loss, which was a key driver in the twentieth century and remains so now (Goulson *et al*., 2015). A series of parasites and diseases infect both wild and managed bees, mostly due to anthropogenic influences. Due to an over-reliance on chemical pesticides targeting the huge agricultural production to feed the burgeoning human population, pollinators are inevitably exposed to a cocktail of agrochemicals. Global climate change is also a major stressor, and it is expected to exacerbate such problems in the future. A nexus of stressors is involved and these do not act alone, making it impossible to anticipate how they will interact; for example, a few herbicides work synergistically rather than additively. Honey bee colony losses and wild pollinator decrease appear to be caused by chronic exposure to a nexus of stressors, while the specific combination appears to differ from place to place.

A study of apple orchards in China's Maoxian county highlighted the consequences of bee decline, where natural insect pollinators are inadequate or non-existent as a result of indiscriminate insecticide usage. Climate change has worsened the loss by causing changes in the weather, such as more frequent showers, cooler temperatures, and gloomy weather during the apple blossom season. This causes asynchrony in flowering, which inhibits pollination by natural pollinators when they are present (Partap and Partap, 2002). Pollination, which was once a free service provided by nature, is no longer available. They have resorted to employing human pollinators for 12-19 USD per person every day, with each pollinating a maximum of 12 plants (Partap *et al*., 2012). To ensure appropriate pollination, it is necessary to boost pollinator intensity in fields and orchards before the alarm bells ring in our neighborhood. Furthermore, roboticists in many labs across the world are building robotic bees to pollinate crops, based on the assumption that natural bees are dwindling and that, humans will soon require a replacement. No matter how far technology advances, we will never be able to replace honey bees with robotic arms to carry out the complex pollination process, which would be costly both economically and environmentally. It is wiser if we begin saving them today to avoid having to regret it later, as the saying goes, ‘Prevention is better than cure’.

Causes of Bee decline:

1. Habitat loss:

Long-tongued bumblebees' ranges have shrunk dramatically as a result of the loss of roughly 97 percent of the floral-rich grass ecology in the United Kingdom throughout the twentieth century (Howard *et al*., 2003; Goulson *et al*., 2008). The loss of bees has been linked to a variety of stresses, some of which are more plausible than others. The loss of habitat is one example. It has directly contributed to bee decline and colony loss, as bees require sufficient floral resources during the adult flight season, which might be short for solitary species or year-round for eusocial species in tropical climates. They also require unaffected nesting grounds, with different species occupying different locations (*e.g.*, cavities underground, hollow-stemmed twigs, burrows in the soil, even abandoned snail shells). The conversion of flower-rich natural and semi-natural habitats into agricultural fields, as well as urbanization, has resulted in significant habitat loss. In gardens, bumblebees and numerous solitary bee species can survive in high numbers, however, in urbanized areas (where roads divide the habitat and traffic cause direct collisions with active colonies), bees are scarce (Bates *et al*., 2011).

2. Pathogens:

Pathogens that cause disease in bees include viruses, bacteria, fungus, protozoans, and other parasites and pathogens. Honey bees, rather than bumblebees or other solitary bees, are the focus of the study, and there is little information on other wild bees. Some disease infects both honeybees and bumblebees, such as deformed wing virus (DWV) and *Nosema ceranae* Fries (Microsporidia: Nosematidae); however, *Paenibacillus* larvae appear to be more host-specific (Graystock *et al*., 2013). The transportation of honey bees across long distance is one of the main causes, which is referred to as "shipping fever." Since the 1960s, *Varroa* mite has spread from Asia to Europe, the Americas, and, most recently to New Zealand (*Varroa* vectors DWV). The mite and the DWV spreads have a synergistic effect, contributing significantly to honey bee colony losses in North America and Europe (Nazzi *et al*., 2012).

In Europe and North America, commercial trafficking of bumblebee colonies for pollination of greenhouse crops like tomatoes is common, and this has resulted in a major loss of bee variety. Tragically, this trade has turned out to be a complete failure. Over one million European bumblebee, *Bombus terrestris* (Linnaeus) (Hymenoptera: Apidae), nests were bred in Europe and shipped to several countries during the 1980s. In North America, the eastern American species *Bombus impatiens* Cresson is raised for this purpose. Unfortunately, it does not appear to be able to raise disease-free colonies, not least because the bees are fed with pollen collected by honeybees, which provides a path for many bee viruses to enter the colony. Parasites like *Nosema bombi* Fantham and Porter, *N. ceranae, Apicystis bombi* (Liu, Macfarlane & Pengelly), and DWV are all common parasites identified in commercial *B. terrestris* colonies (Graystock *et al*., 2013).

3. Introduction of exotic honey bee species for managed pollination:

Wild bumblebee populations have been demonstrated to be severely impacted by non-native infections or pathogen strains associated with these colonies. Species can become extinct merely as a result of ignorance. Despite the presence of native *Bombus* species, the Chilean government deliberately introduced *B. terrestris*, which soon expanded across southern South America. The introduction of *B. terrestris* in the area appears to have resulted in the rapid extinction of the native *Bombus dahlbomii* Guerin-Meneville, at a rate that can only be explained by pathogen spillover. Although the parasite responsible for the invasion has yet to be identified with certainty, both *Apicystis bombi* (Liu, Macfarlane & Pengelly) and *Crithidia bombi* Leger have been discovered to be common among invading species (Schmid-Hempel *et al*., 2013). It is gratifying to note that the large-scale introduction of bumblebees from other countries has not been on hold in India owing to its perceived impact on native bumblebee diversity in the country (Uma Shankar, 2014).

4. Injudicious use of pesticides:

Herbicides, on the other hand, allow farmers to plant near-pure monocultures, but their use diminishes the availability of flowers for pollinators, making farmland uninhabitable for bees (Goulson *et al*., 2008). Sanchez-Bayo and Goka (2014) anticipated that three neonicotinoids (thiamethoxam, imidacloprid, and clothianidin) and two

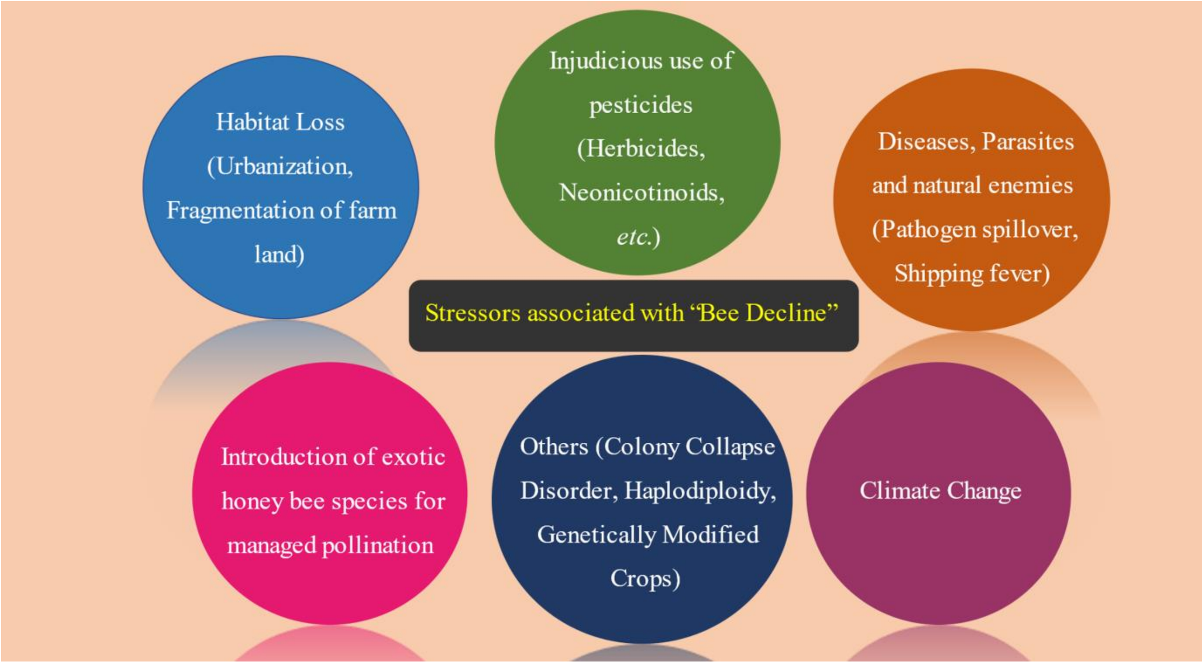

Plate 1: Causes of Bee decline

organophosphates (phosmet and chlorpyrifos) pose the greatest harm to honey bees on a worldwide scale out of 161 pesticides used. Chemical insecticides continue to remain a major threat to domesticated and wild bees ever since the introduction of high-yielding varieties of crops in the 1960s. Many regulations are in place to check the indiscriminate use of insecticides which would potentially endanger the bee pollinators. A lot of concern has been raised about the possible impact of neonicotinoids on bee health. Several reports allude to the colony collapse to the widespread use of neonicotinoids in crops (Krupke *et al*., 2012; Han *et al*., 2010).

Neonicotinoids are widely used for seed treatments because they are systemic within plants, spreading through plant tissues and into pollen and nectar of blooming crops like canola (Cutler and Scott-Dupree, 2007); sunflower (Higes *et al*., 2010); corn (Genersch *et al*., 2010). They're also water-soluble yet exceedingly persistent in soil and soil water, therefore they've been found in biologically significant concentrations in pollen and nectar from wildflowers near crops (Krupke *et al*., 2012). Both honey bees and bumblebees have shown sublethal symptoms of neonicotinoid exposure, including reduced learning, foraging, and homing abilities, all of which are critical for bee survival (Han *et al*., 2010). In bumblebees, however, exposure to field-realistic levels of imidacloprid significantly slowed colony growth and reduced queen production by 85 per cent (Whitehorn *et al*., 2012).

Bee decline may be caused by a complex interplay of stresses; embryonic exposure to neonicotinoid insecticides makes honey bees more vulnerable to the invading disease, *N. ceranae* (Wu *et al*., 2012). Colony Collapse Disorder (CCD) is also a result of the stresses combined. Neonicotinoids are highly potent neurotoxic insecticides, and the finding that they are present in 75% of the honey samples collected from around the world (Mitchell *et al*., 2017) demonstrates that bees (and by implication many other insects) are routinely exposed to them.

5. Climate change:

The most prevalent man-made pollution, of course, is greenhouse gas emissions, which cause climate change. Some insects' ranges have begun to move as a result of climate change, with European and North American bumblebees disappearing from the southern borders of their range and adopting higher elevations in mountainous areas (Pyke *et al*., 2016). There is also evidence that the phenology of some herbivorous and pollinating insects are becoming decoupled from their host plants. For example, in Colorado, some highland plants are now blooming before bumblebees have emerged from hibernation when they did not previously (Pyke *et al*., 2016). Low electromagnetic fields, such as those formed by high voltage cables, have been found to impair the cognitive capacities of honey bees, and it has been claimed that this may have led to bee colony losses and, more broadly, could disrupt insect navigation and dispersal (Shepherd *et al*., 2018).

Adoption and mitigation measures:

Conservation is an anthropocentric pursuit; it requires integrating social and ecological understandings and greater coordination to advance insect pollination conservation practices and policies fit for Anthropocene. Humans are the major beneficiaries of the ecological services rendered by bees. Ironically, bees have been viewed as a conditional danger – annoying if provoked- and education about bees vanishes fear, and increases interest and willingness to protect them. There is no silver bullet in determining bee-friendly practice adaption. A key strategy to support native and managed bees is through diversified practices such as planting and maintaining permanent pollinator habitats. Diversification also serves as an adaptive capacity by enhancing the foraging range of the wild and non-domesticated bees which comprises ecological engineering and habitat management. Strips of cover crops, hedgerows, or wildflower strips can increase wild pollinator diversity and visitation. Moreover, the availability of such floral resources regularly ensures the honey bee's health. Incentivizing the beekeepers and their neighbors to adopt diversified bee-friendly management practices such as forage planting, artificial nesting sites, and reduced pesticide use directly increase food security and pollinator health. Progressive beekeepers are encouraged through initiatives such as USDA's EQIP (Environmental Quality Incentive Program) and the non-profit Project Apis m's "Seeds for Bees" cost-free program for cover crop planting in the United States. Urban populace around the world is leaning toward sustainable living, and it can be contemplated by the construction of buildings using hollow bricks, and 'bee hotels' are more sought after to encourage solitary bees for nesting.

In the absence of sweeping international agreements aimed at pollinator conservation, national, sub-national, and municipal governments are developing policy approaches to address insect pollinator conservation. Many policies from legislative bodies include- officially designating

"Pollinator Day" or "Pollinator Week", in many others "Bee Day" to raise awareness; tightened beekeeping standards to manage pathogens; creating a task force to update pest management approaches (pesticide use); funded research and monitoring for managed bees and native insect pollinators. In India, there are Centers for Excellence for beekeeping especially, AICRPs, and IBDCs throughout the country to carry out region-specific research and disease management. There are nascent efforts to goad in international policy. Emergent from the United Nations Convention on Biological Diversity Conferences, in 2018, nations began coordinating national strategies for pollinator protection through a coalition of willing pollinators called "Promote Pollinators" (~ 25 members). Coalition members share methods for conservation and tactics for instigating policy.

Apart from biotic and abiotic factors, bees' decline needs to be addressed based on their genetics, with special emphasis on haplodiploidy, as the haplodiploidy and complementary sex determination affect genetic parameters which are pertinent to the viability of bees. Hymenopteran shows the genetic effect of population fragmentation over other insect orders and the reduced population size due to haplodiploidy. Because in one generation there are only ¾ gene copies in haplodiploid organisms compared to diplo-diploid ones, haplodiploid hymenopterans will generally have less effective population size which reduces the heterozygosity and calculation of population size is highly complex due to unusual sex ratio (Packer and Owen, 2001). The production of inviable or sterile diploid males, a necessary by-product of complementary sex determination, is a large threat to the short-term viability of small bee populations. Although, happening of inbreeding depression by dominance and overdominance is lower in haplodiploids compared to diploids, it still reduced the fitness and viability of small bee populations which shows adversity in conservation (Zayed, 2009).

Conclusion:

In general, the loss of nectarivorous bees would result in the significant plummeting of forest and agro-biodiversity, resulting in skyrocketing food prices. Moreover, many floral species are dependent on honey bees for pollination and many local communities also depend on honey and wax as a source of their livelihood security. Bees, which are critical to the survival of the human race, are on the decline majorly due to the use of pesticides and habitat loss. Conservation prescriptions appear attainable; they emphasize managing rural and urban lands to maximize forage for insect pollinators and curb agrochemical-pollinator interactions.

Abbreviations:

AICRP-All India Coordinated Research Project

CCD: Colony collapse disorder

DWV: deformed wing virus

EQIP- Environmental Quality Incentive Program

IBDC-Integrated Bee Development Centers

NRC: National Research Council

USDA: United States Department of Agriculture

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Could electromagnetic radiations have any effect on insects and their behaviour?

Mayank Kumar and A K Pandey

Electromagnetic radiations (EMR), are the radiations that travel through space by carrying electromagnetic radiant energy. These radiations vary in strength from low energy to high energy. The energy is spread all around in many forms, such as radio waves, microwaves, X-rays etc. Sunlight is also a form of EM energy, but visible light is only a small portion of the EM spectrum, which contains a broad range of electromagnetic wavelengths.

In classical physics and modern quantum theory, EMR is flow of energy or photon at the universal speed of light via free space or through a material medium in the form of electric and magnetic fields which generally characterized by its intensity and frequency of time variation to the electric and magnetic fields. It has created due to electrically charged particles undergoing periodic change of electric or magnetic field. Due to their different sources of emission and effects on matter, they are called by different names. However, these EM waves carry energy, momentum and angular momentum away from their source particle and can impart those quantities to matter with which they interact.

Likewise, EMR also interacts with arthropods life. It always remains to be a contradictory with insects. Most of the advance technologies like electronic gadgets, mobile phones emit electromagnetic radiations. Their harmful effects have already warned humans of their safety but somehow at present they are still the fastest growing industries. At present, global biodiversity declining very fast rate among which arthropods are major one. The arthropod biodiversity is declining at the rate of 1-2% per annum. Among the arthropods, bees and pollinators are highly affected by EMR (Atwal, 2018) which resulted in tremendous disturbances of food web of both terrestrial and aquatic ecosystems. This has also resulted into a wide gap in food chains for which at now humans do not have any alternative way to fill up those food chain gaps. Such facts undoubtedly emphasize to initiate the strategies to act on reduction of electromagnetic radiations for preservation and maintenance of not only arthropods but for whole biodiversity along with humans for their healthy survival.

EMR and insects:

In mid-20th century, there is been abrupt increase in the emission of EMR (radio waves, microwaves, infrared, visible light, ultraviolet, X-, and gamma radiation) in the environment (Table 1). Now days, android phones have also entered in this category with advance cellular system i.e. 5G. The 5G utilizes frequencies up to 120 GHz (Thielens *et al*., 2018). Their adverse effects have already been seen on vertebrates (Humans, mice, birds) and up to limited extent on arthropods especially bees (Atwal 2018).

Table 1. EMR and their impact on organisms.

Sl. No.	Radiation	Energy type	Frequency	Wavelength	Impact on organisms	Example
1	Extremly low frequency (ELF)	Non - ionizing	$10\text{-}10^{6}$Hz	$10^{7}\text{-}10^{3}$m	Safe and beneficial in appropriate dosage	Power lines, electrical wiring
2	Very low frequency (VLF)	Non – ionizing	$10^{6}\text{-}10^{7}$Hz	$10^{3}\text{-}10^{1}$m	Safe and beneficial in appropriate dosage	Radio navigation
3	low frequency (LF)	Non – ionizing	$10^{7}\text{-}10^{8}$Hz	10^{1}-1m	Safe/ low danger	Radio, TV
4	Radio frequencies	Non – ionizing	$10^{7}\text{-}10^{12}$Hz	$1\text{-}10^{-1}$m	Safe/ low danger	Radio, television, Mobile phones
5	Microwaves	Non – ionizing	$10^{10}\text{-}10^{12}$Hz	$10^{-1}\text{-}10^{-3}$m	Danger	wireless network, routers, satellite and space craft communication
6	Infra-red	Non – ionizing	$10^{14}\text{-}10^{12}$Hz	$10^{-4}\text{-}10^{-5}$m	Danger	Electrical heater, cooker, remote control, thermal imaging cameras
7	Visible	Non – ionizing	$10^{14}\text{-}10^{15}$Hz	10^{-6}m	Safe and beneficial in appropriate dosage	Li-Fi, underwater communication,
8	Ultraviolet	ionizing	$10^{15}\text{-}10^{17}$Hz	$10^{-7}\text{-}10^{-9}$m	Extremely Harmful	Lamps, TVs, tablets, computer etc.
9	X-rays	ionizing	$10^{16}\text{-}10^{20}$Hz	$10^{-8}\text{-}10^{-12}$m	Extremely Harmful	X ray machine, electron microscope, radiography etc.
10	Gamma rays	ionizing	$>10^{19}$Hz	$>10^{-11}$m	Extremely Harmful	Nuclear reactors,

In May 2011, World Health Organization (WHO) mentioned radiation with the frequency range of 30 kHz to 300 GHz in Group 2B and can be a 'possible' human carcinogen, (Baan *et al*., 2011; IRAC, 2013), but somehow, they are being ignored. The maximum studies of EMR have been conducted on two insects i.e. the fruit fly (*Drosophila melanogaster*) and honey bees (*Apis mellifera*). In fruit fly, EMR shows developmental delay and reproductive failure while in honey bees they were found degrading the colony strength and oviposition rate. Due several limitations like methodologies, equipped laboratory, rearing techniques and time limitation etc. researchers are unable to obtain its effects on other insects.

Some insects have found capable to recognize EMR for example honey bees. They are able to detect magnetic field and EMR of same magnitude for orientation, navigation and foraging (Venbergen *et al*., 2019). Sometimes for intraspecific and interspecific (plant-pollinator) communication, they also utilize EMR (Clarke *et al*., 2013; Greggers *et al*., 2013). But ultimately it could disturb the physiological functions even more in some cases affects its survival. At now, each species on this earth have some relationship with environment for some special functions like pollination, food chain, nutrient cycle etc. but unfortunately, climate change, pollutions including EMR, deforestation, urbanization etc. created an unresolved threat for biodiversity conservators to preserve them in present scenario for future generations.

Effect of EMR on insects:

At present very few researches were conducted on insects to observe the effect of higher radio Frequency and EMR on them (Fig. 1).

According to Thielens *et al*., (2018) when insects are exposed to EM fields, it is partially absorbed by their body based on their frequency. If frequency is at or above 6 GHz, it will increase the general absorption of RF power. Their simulations with insects, showed a shift of 10% in the incident power density to frequencies above 6 GHz that would lead to an increase in absorbed power between 3–370%. These results were the outcome of their research on four insects i.e. *Tetragonula carbonaria* (Australian Stingless Bee), *Apis mellifera* (Western Honeybee), *Schistocerca gregaria* (Desert Locust) and *Geotrupes stercorarius* (Beetle) with the help of micro-CT imaging to develop realistic model. These shifts ultimately could bring the changes in insect behaviour, physiology, and morphology over time due to increased body temperatures, from dielectric heating. Similarly, in 2020 they conducted an in-situ experiment in Belgium exclusively on economic insect *Apis millifera* and on its different cast i.e. two workers, one drone, one larva and a queen. They exposed the five-bee model to different frequency of EMF i.e. from 0.6 GHz up to 120 GHz. The average absorbed radio-frequency power increases by factors 16 to 121. It concludes that 10% of the incident power density would shift the frequencies up to higher than 3 GHz. It would lead to an increase of this absorption between 390–570%. In the series of experiment, Francis, 2020 showed the impact of EMR released from cell phones and their base stations on organisms. He highlighted the negative effect of these EMR on plants, insects, birds and some other animals. According to him radiations from mobile base stations and from cellular mobile is almost identical i.e. Global System for Mobile communication (GSM) 900 or 1800, except that it is simply more powerful. GSM 900 mobile phones emit radiations with a frequency between 890 and 915 MHz, while base stations emit radiations

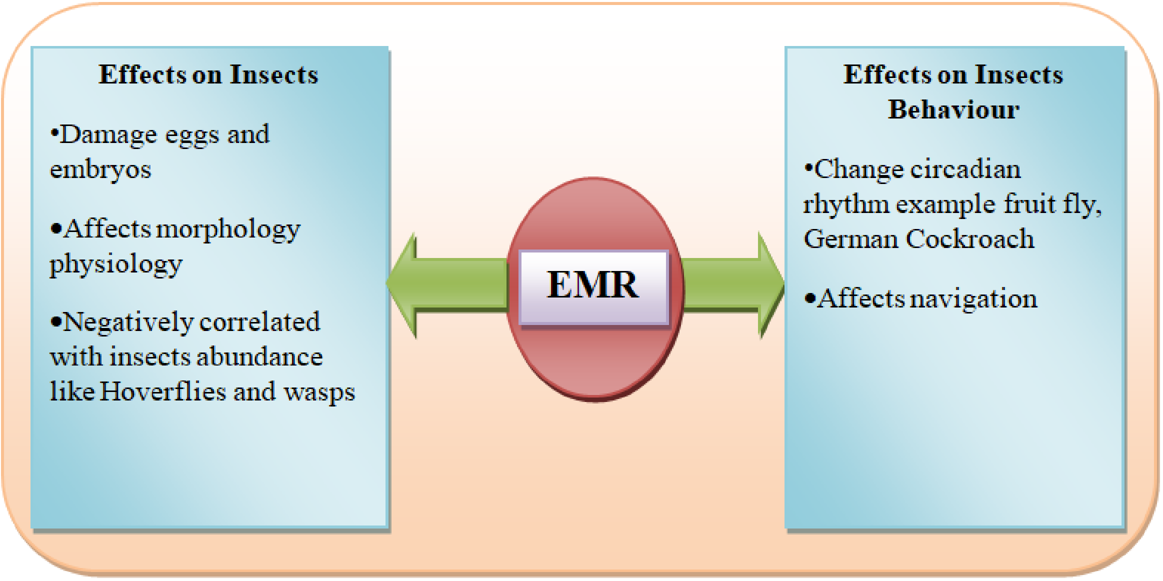

Fig. 1: Effects of EMR on Insects and their behaviour.

between 935 and 960 MHz. Overall, stronger the field, greater is the undesirable impact of the electromagnetic field on life. It could disrupt birds' and bugs' orientation and movement, and affect plant metabolic health, bug life warns. The microwaves (300 MHz to 300 GHz) emitted by cell phone towers and handsets has been found to be responsible for damaging eggs and embryos of sparrows.

In field realistic settings, to observe EMR impact on insect's abundance, Lazaro *et al.*, 2016conducted a small experiment across two Aegean islands. High frequencies (800–2600 MHz) were distributed there. His findings showed that EMR can affect the insect abundance especially wild pollinator. Their analysis revealed a complex correlation between the variable anthropogenic electric field (range 0.01–0.67 $V \cdot m^{-1}$) and insect abundance, but these were contingent on the insect taxon and sometimes varied with geographical (island) location. Greater exposure to EMR was related negatively to hoverflies and wasps abundance, positively to underground nesting wild bees and bee flies and uncorrelated to butterflies abundance. EMR did not show any effect on species richness of wild bees and hoverflies.

Effect of EMR on insect behavior:

Insect behaviour is the outcome of various actions in response to a stimulus or to its environment. It covers a wide range of activities like movement, feeding, learning, reproduction etc. but EMR may hamper them. It could easily understand from the findings of following researchers. Early research by Koschnitzke *et al.*, (1983) on insects (Chironomidae) against different frequencies (64.1–69.1, 67.2, 68.2 GHz) shows that millimeter waves of a power density less than 6 m W /cm 2 exert a non-thermal influence on the chromosomes of our eucaryotic system. All frequencies, which using power densities <6 mW/cm2, were found in responsible to cause a reduction in the size of a particular area of the insect chromosome. Another research was conducted by Fedele *et al.*, (2014) on circadian clock of *Drosophila melanogaster* to observe the effect of electromagnetic field on cryptochrome (CRY) photoreceptor. Basically, these cryptochrome are utilized by *Drosophila melanogaster* to navigate natural EMF during migration when activated by light. From his study they conclude thathCRY2 is blue light-responsive. Deletion of the CRY C-terminus i.e. blue-light sensitive photoreceptor cryptochrome markedly attenuates the EMF-induced period, whereas the N-terminus underlies the hyperactivity. Ultimately, hCRY2, transformants can detect the EMFs.

Likewise, in the way Bartos *et al*., (2019) finds their results on German cockroach (*Blatella germanica*) by utilizing remarkably weak intensities of man-made radiofrequency (RF) i.e of in nanotesla range. They found that weak broadband RF fields can affect the circadian rhythm of the German cockroach (*Blatella germanica*). Their data shows that the circadian rhythm of the insect species *B. germanica* is sensitive to both static MF and RF fields in a light-dependent manner. Some other also noted their impact on physiology. Weak broadband RF noise impacts the insect clock system which is comparatively more sensitive to RF than to static MFs.

At now there are very little evidence of exposure to EMR that leading to any damage or harmful effect on individual's development, reproduction of few insect species like flying insects (Wan *et al*., 2014; Wyszkowska *et al*., 2016). At present most, convincing finding of EMR are with birds and mammals, (Engels *et al*., 2014; Malkemper *et al*., 2018). Overall, the magnetic sense of invertebrates appears to be affected by EMR in the MHz-range although the extent to which arthropods especially insects are dependent on their magnetic sense for successful navigation remains unknown.

Conclusion:

In vertebrates, present scenario reveals that Aves (birds) and mammals are highly affected with EMR (Engels *et al*., 2014). It may be due to the close association of birds with mammals in classification. But one cannot neglect their side effects on invertebrates' organisms like insects. Where these EMR may hamper their abundance, physiology functioning, behavioural aspect and ultimately their survival. But due to unavailability of methodologies, proper techniques, and several limitations by environmental agency one has failed to calculate out those impacts. So, further research on this area must bring some unbelievable results which can surely bring some changes in science of insects. Till date, studies are very less on EMR impact on insects to reach at any conclusion but their possible outcomes may help to understand the side effects of these EMR on these small creatures.

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Wondered to know the threat for wonder tree

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Neem (*Azadirachta indica* A. Juss.), popularly called as "Indian wonder tree", is known for its medicinal values as anti-malarial, anti-fungal, anti-viral and anti-carcinogenic effects. This is one of the most popular plant products being utilized in crop protection for the control of crop-pests. Apart from its various commercial formulations available in the market (Azadirachtin 300 ppm, 1500 ppm, 10000, 15000 ppm), its extract, NSKE @ 5 % is commonly used against crop-pests. The neem tree is being attacked by insects is a rare situation particularly the stem borers. However, it's surprising to know that in the present situation thousands of neem trees across Karnataka state are attacked by a mirid bug known as "Tea Mosquito Bug" (TMB), *Helopeltis antonii* (Fig. 1). A plant having pesticidal property is being attacked by a pest is really a wonder. TMB normally attacks fruit crops and few plantation crops; however, neem was reported as an alternative host for TMB (Saroj *et al*., 2016). It normally does not pose any threat to neem, but its pestiferous nature in the recent past can be attributed to climate change induced favourable weather conditions (Kalyanasundaram, 2016). Hence, the information pertaining to TMB in terms of host range, biology, nature of damage and management is needed to bring awareness among the scientific and, farming community and common people.

Host Range: Three species of *Helopeltis*, *viz*., *H. antonii*, *H. bradyi* and *H. theivora* were recorded in India, where *H. antonii* is the dominant species (Sundararaju and Bakthavatsalam, 1994). *Helopeltis antonii* with a life cycle of 25-32 days is also found in plants like cinchona (*Cinchona* spp.), persian neem and annatto (Fletcher, 1914); mahogany (Fletcher, 1914; Rao 1915); cashew and guava (Ayyar, 1940; Puttarudriah 1952); avocado (Puttarudriah, 1952), apple and grapevine (Puttarudriah and Appanna, 1955); cotton (Puttarudriah, 1958); cocoa *(Theobroma cocoa L.)* (Abraham and Remamony, 1979); camphire (*Lawsonia alba* Lam.) (Sundararaju, 1984); drumstick (*Moringa oleifera* Lam.) (Pillai *et al*., 1979); rose apple, mango, all spice and black pepper (Piper nigrum L.) (Devasahayam and Nair,1986); poria tree (*Thespesia populnea* L.) (Sundararaju and Baktavatsalam, 1994); *Ailanthus excels* Roxb. (Satapathy, 1993; Sundararaju, 1996); ber (*Zizyphus mauritiana* Lam.), Indian gooseberry (*Emblica officinalis* L.), cotton (*Gossypium barbadense* L. and *G. hirsutum* L.), cowpea and Compositae weed plant (*Lactuca runcinata* DC.) (Sundararaju, 1996); neem (Onkarappa and Kumar 1997; Sundararaju and Sundarababu,1999); Singapore cherry (*Muntingia calabura* L.) (Srikumar and Bhat, 2013); *Annona* spp. (Venkata Rami, 2009).

Biology: The eggs of TMB are white, ovo-elongate, laterally compressed measuring about 1.0 to 1.31 mm long (Ambika and Abraham, 1979). Two unequal extra-chorionic processes arise from the anterior end of the eggs measuring nearly 0.29-0.67 mm in length. The eggs are embedded in plant tissue singly or in small groups usually with the operculum and extra chorionic processes exposed. The fecundity of *H. antonii* on cashew varies from 10 to 41 eggs (Pillai *et al.,* 1984) and 28 to 35 eggs (Ambika and Abraham, 1979). *Helopeltis antonii* lays its eggs primarily on the young shoots, inflorescence stalks and tender nuts of cashew, but sometimes accepts the petioles and ventral midribs of leaves (Ambika and Abrahamm, 1979). On guava, *H. antonii* lays eggs primarily on ventral midribs of leaves, flower buds, and pea-sized guava fruit singly or in groups (Sundararaju and Sundarababu, 1999). Incubation period of *H. antonii* varies among hosts *viz.*, neem (8-9 days), guava (9-10 days) and cashew (7-8 days) as reported by Sundararaju (1996). It has five nymphal instars that vary in size, duration, colour and development of body parts. Nymphal developmental periods of 15.3 ± 0.82 days were recorded for *H. antonii* on neem; 20.24 ± 1.79 days on guava and 12.60 ± 0.50 days on the cashew (Sundararaju, 1996). The longevity of *H. antonii* adults varied from 7 to 46 days (Stonedahl, 1991; Sundararaju, 199; Srikumar and Bhat, 2011).

Damage symptoms: It sucks the plant sap from tender shoots of neem causing degeneration and injects poly-phenol oxidase from their salivary glands (Mandal, 2000) thereby, complete drying of affected branches is noticed (Fig. 2). Typical feeding damage by *H. antonii* appears as a discoloured necrotic area or a lesion around the point of entry of the labial stylets inside the plant tissue leading to the destruction of plant cells. The infestation of inflorescence results in “blossom blight” (Devasahayam and Nair, 1986). When they puncture the tissues, a gum oozes out of the holes as depicted in Fig 3.

Fig. 1: Tea mosquito bug adult, *Helopeltis antonii*

Severity of TMB: Thousands of neem trees were found infested by mirid bug during September to December, 2021 in several districts of Karnataka *viz.,* Raichur, Koppal, Yadgir and Ballari. Even the invasion of this bug was also been reported from Rayalaseema region of Andhra Pradesh (Kurnool, Anantapur, and parts of Kadapa district) and Telangana (Mahaboobnagar and Hyderabad) during November, 2021. The pest’s infestation on cashew started from October/November pronounced more during December to March with the population reaching peak in January during which the trees were in full bloom with flowering and fruiting. Later the population subsided during the monsoon period (Sundararaju, 2005). Kalloor *et al*. (2020) reported after the northeast monsoon shower ceased at Mettupalayam, Coimbatore, population of TMB started infesting neem during October-November, 2019. The bugs were active in neem until April, 2020 at varied levels of intensity and the pest population reached its peak during January, 2020. The highest

Fig. 2: Drying of infested branches due to tea mosquito bug

Fig. 3: Oozing of gum from feeding punctures by tea mosquito bug

number of TMB was recorded (9.60 per three terminal shoots per tree) during the third standard week of 2020 and the lowest numbers were recorded during 15th standard week 2020 (2.00 per three terminal shoots per tree). Wind velocity, maximum temperature, rainfall, relative humidity, sunshine hours and evaporation rates were found negatively correlated with the population of TMB. While, minimum temperature was positively correlated with the population abundance of TMB (Kalloor *et al*., 2020).

Management: Collect and destroy the damaged plant parts followed by spraying the trunks, branches, foliage, and inflorescence early in the morning or late in the evening hours is recommended for efficient control with flonicamid 50% WP @ 0.50 g or dinotefuron 20 % SG @ 0.30 g or acephate 75% SP @ 1.0 g or thiamethoxam 25% WG @ 0.30 g or profenofos 50 % EC @ 2ml or acetamiprid 20% SP @ 0.6 gm per litre (Radhika, 2020; Padmaiah, 2021). Bio-control agents particularly *Telenomus* spp, an efficient egg parasitoid on tea mosquito eggs (Kalyanasundaram, 2016). Research is needed to figure out how the bugs managed to evade the insect-repellent azadirachtin, a chemical molecule found in practically every part of the neem tree.

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Diversity of insect pests and natural enemies of rice bean (*Vigna umbellata*) from Manipur

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Abstract: Rice bean is considered as underutilized grain legume crop in India and is hampered by several insect pests. It has a good nutritional value and regarded as one of valuable crop in Manipur. Recently a survey was undertaken to document insect pests and natural enemies of rice bean in Manipur. The results revealed that *Paracoccus marginatus* having the highest relative abundance among the insect pests species (10.91%), followed by *Aleurodicus disperses* (10.81%) and in case of natural enemies, *Solenopsis* sp (15.19%) having the highest relative abundance followed by *Oxyopes* sp (10.91%).

Key words: Rice bean, *Paracoccus marginatus, Aleurodicus disperses, Solenopsis* sp, *Oxyopes* sp

Rice bean (*Vigna umbellata*) (Thunb.), belonging to the family Fabaceae is considered as underutilized grain legume crop in India. It is mostly cultivated in hilly areas in India and parts of Southeast Asia. Rice bean majorly grown as intercrop or mixed crop with maize (*Zea mays*), sorghum (*Sorghum bicolor*) and cowpea (*V. unguiculata*). It is distributed from Southern China through the north of Vietnam, Laos and Thailand into Burma and India (Tomooka *et al.*, 1991). In India, rice bean is cultivated mainly in the Himachal Pradesh and Uttaranchal and NE hills (Manipur, Assam, Arunachal Pradesh, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura), as well as in Central India in Madhya Pradesh and Chhattisgarh. It can be full-grown in diverse conditions and familiar among farmers for its wide adaptation and production even in marginal lands and drought-prone sloping areas. These species are also termed as 'orphan' crops as there is scanty research and development, and as such there is no scientific knowledge about them (Eyzaguirre *et al.*, 1999). The wild forms are mainly cultivated in Kerala, Eastern and Western Ghats to the Himalayas and there is unlimited variability, particularly in the NE hill region. The wild types of rice bean occur in natural and disturbed habitats and forest clearings, and most of the plants with small seeds. Bisht *et al.*, (2005) reported that within-species variation in rice bean was higher than in related species. It is well adjusted to the humid tropics and does well on many soil types, with most varieties are highly photoperiod sensitive, late flowering and have strong vegetative growth. It can adapt to drought, excessive rain, and resistance to viral, bacterial and fungal diseases, good anti-inflammatory properties (Singh *et al.*, 2020). It is a rich source of protein and contains high levels of essential fatty acids, essential amino acids, minerals and vitamins

(Mohan & Janardhanan, 1994). Although rice bean is considered as underutilized crop, it has a good nutritional value and regarded as one of valuable crop in Manipur. Rice bean is hampered by several insect pests in Manipur and there is scanty research about the insect pest complex in Manipur. Hence, it is important to study the relative abundance, pest status and crop stage of insect pest and natural enemies of rice bean in Manipur.

Materials and Method:

The study was conducted at Farmers field, Imphal District, Manipur, India 24º47'26.3"N 93º55'22.2"E during 2020-21. The experiment was laid out in a Randomized Block Design (RBD). A population of insect pests and natural enemies on ricebean was surveyed during 2020-21 by regular observation of weekly intervals after transplanting with10 randomly plants for the experiment. The specimen which came along were collected by hand picking and sweeping net and were brought for identification. The microscopic insect specimen was observed by microscope (Leica stereo zoom, SZM, S9i).

Relative Abundance (RA) of a species =

$$\frac{\text{No. of individuals of the species}}{\text{No. of individuals of all species}} \times 100$$

Results and Discussion:

Occurrence of insect pests and natural enemies in rice bean crop:

The occurrence of insect pests and natural enemies of rice bean was studied from different crop stages i.e. vegetative, flowering + fruiting (reproductive) and maturity. Study revealed that seven insect pest species and six species of natural enemies were observed at different stages of crop (Table 1&2).

Among seven insect pest species four species such as *Aleurodicus dispersus* (Russel), *Aphis gossypii* (Glover), *Bemisia tabaci* (Gennadius), *Paracoccus marginatus* (Williams and Granara de Willink) belong to the order Hemiptera, *Phragmatobia fuliginosa* (Linnaeus) and *Phalanta phalantha* (Drury) belong to the order Lepidoptera, *Ophiomyia phaseoli* (Tryon) belong to the order Diptera. Among six species of natural enemies two species viz., *Coccinella transversalis* (Fabricius) and *Chilocoris nigrita* Fabricius belong to the order Coleoptera, *Oxyopes* sp (Latreille) belong to the order Araneae, *Crocothemis servilia* Drury belong to the order Odonata, *Solenopsis* sp Buren belong to the order Hymenoptera and *Rhynocoris iracundus* Latreille belong to the order Hemiptera were recorded as natural enemies in rice bean ecosystem (Table 1). The order Hemiptera and Coleoptera is found to be the most dominant insect pest and natural enemies which is similar with the finding of (Thokchom *et al.,* 2019). *Aleurodicus dispersus* was first time reported from brinjal in Manipur (Thokchom and Akoijam, 2022). Also, *A. disperses* will be the first report on rice bean in Manipur. It was observed from November to December during reproductive stage (Table 1). Nymphs and adults of *A. gossypii* were observed during vegetative stage from October to January (Table 1). *Ophiomyia phaseoli* was found during reproductive stage from February to March (Table 1). *Phragmatobia fuliginosa* was seen during reproductive stage from March to April.

Table 1. List of insect pests of rice bean during 2020-21

Insect Pest	Scientific name	Order	Family	Damaging stage	Crop stage	Period of activity	Pest status	Relative abundance %
Spiralling whitefly	*Aleurodicus dispersus* Russel	Hemiptera	Aleyrodidae	Nymph & Adult	Reproductive	Nov to Feb	High	10.81
Aphids	*Aphis gossypii* Glover	Hemiptera	Aphididae	Nymph & Adult	Vegetative	Oct to Jan	High	9.32
Bean fly	*Ophiomyia phaseoli* Tryon	Diptera	Agromyzidae	Adult	Reproductive	Feb-March	Low	5.19
Ruby Tiger moth caterpillar	*Phragmatobia fuliginosa* Linnaeus	Lepidoptera	Erebidae	Larva	Reproductive	March-April	Low	4.32
Leopard butterfly	*Phalanta phalantha* Drury	Lepidoptera	Nymphalidae	Larva	Reproductive	Feb-March	Low	4.19
Whitefly	*Bemisia tabaci* Gennadius	Hemiptera	Aleyrodidae	Nymph & Adult	Vegetative	Oct to Jan	High	9.58
Mealy bug	*Paracoccus marginatus* Williams and Granara de Willink	Hemiptera	Pseudococcidae	Nymph & Adult	Reproductive	Dec to Feb	High	10.91

Table 2. List of natural enemies of rice bean during 2020-21

Insect pest	**Scientific name**	**Order**	**Family**	**Crop stage**	**Period of activity**	**Relative abundance %**
Transverse Ladybird beetle	*Coccinella transversalis* Fabricius	Coleoptera	Coccinellidae	Vegetative	Nov - Feb	10.81
Ladybird beetle	*Chilocoris nigrita* Fabricius	Coleoptera	Coccinellidae	Reproductive	Jan-Feb	1.19
Dragon fly	*Crocothemis servilia* Drury	Odonata	Libellulidae	Reproductive	Oct - March	6.13
Assassin bug	*Rhynocoris iracundus* Latreille	Hemiptera	Reduviidae	Reproductive	Feb- March	1.39
Spider	*Oxyopes sp* Latreille	Araneae	Oxyopidae	Reproductive	Feb - March	10.91
Fire Ants	*Solenopsis sp* Buren	Hymenoptera	Formicidae	Vegetative	Nov-April	15.19

Larvae of *P. phalantha* were observed from February to March during reductive stage (Table 1). *B. tabaci* was seen from October to January during vegetative stage (Table 1). Nymph and adult *P. marginatus* were found from December to February during reproductive stage (Table 1). The highest relative abundance of insect pests was recorded for *Paracoccus marginatus* (10.91%), followed by *Aleurodicus disperses* (10.81%), *Bemisia tabaci* (9.58%), *Aphis gossypii* (9.32%), *Ophiomyia phaseoli* (5.19%), *Phragmatobia fuliginosa* (4.32%) and *Phalanta phalantha* (4.19%) (Table 1). Natural enemies such as *C. transversalis* were observed during vegetative stage from November to February (Table 2). *Chilocoris nigrita* was seen during reproductive stage from January to February (Table 2). *Crocothemis servilia* was observed during reproductive stage from October to March (Table 2). *Rhynocoris iracundus* and *Oxyopes* sp were seen during reproductive stage from February to March (Table 2). *Solenopsis* sp was seen during vegetative stage from November to April (Table 2). The highest relative abundance of natural enemies was found for *Solenopsis* sp (15.19%) followed by *Oxyopes* sp (10.91%), *Coccinella transversalis* (10.81%), *Crocothemis servilia* (6.13%), *Rhynocoris iracundus* (1.39%) and *Chilocoris nigrita* (1.19%) (Table 2). The results were similar with the findings of Kumar *et al.*, 2017 who reported aphids, jassids, pod borer and coccinellids as the insect pests and natural enemies in mungbean with the highest relative abundance in aphids and coccinellids.

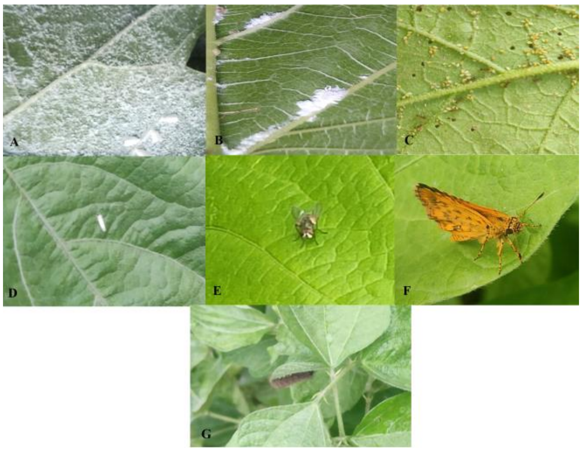

Fig. 1. Insect pests of rice bean: A. *Aleurodicus dispersus* Russel, B. *Paracoccus marginatus* Williams and Granara de Willink, C. *Aphis gossypii* Glover, D. *Bemisia tabaci* Gennadius, E. *Ophiomyia phaseoli* Tryon, F. *Phalanta phalantha* Drury and G. *Phragmatobia fuliginosa* Linnaeus.

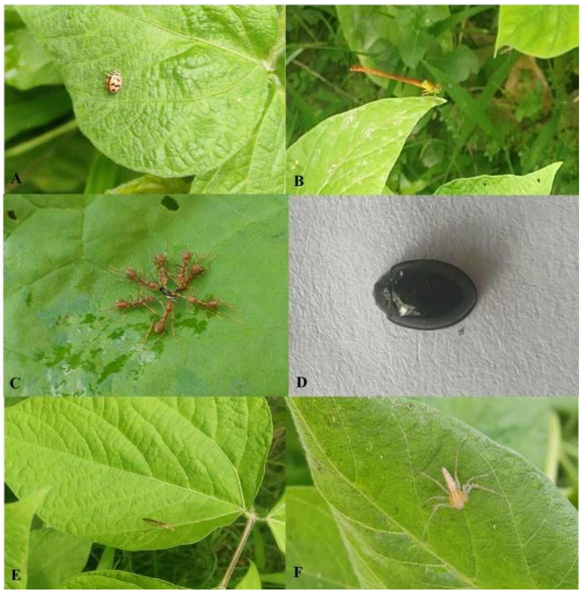

Fig. 1. Natural enemies *recorded in* rice bean: A. *Crocothemis servilia* Drury, B. *Coccinella transversalis* Fabricius, C. *Solenopsis* sp Buren, D. *Chilocoris nigrita* Fabricius, E. *Rhynocoris iracundus* Latreille, and F. *Oxyopes* sp Latreille.

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Gallfly, *Trioza fletcheri* minor Crawford and its management in Tasar food plants

B Thirupam Reddy, Hanamant Gadad, Vishal Mittal, J Singh and K Sathyanarayana

Trioza fletcheri is a subtropical plant louse, induces leaf galls on at least five species of *Terminalia* in the Indian subcontinent. Unlike a majority of gall-inducing psylloids that are generally host and site specific (Hodkinson, 1984), *T. fletcheri* is known not only from the leaf galls of *T. tomentosa* and *T. arjuna*, but also from those of *T. catappa* Linn., *T. paniculata* Roth, and *T. tomentosa* X *T. arjuna* hybrids (Mathur, 1975). Among these *T. tomentosa* and *T. arjuna* are important and commercially exploited host plants of tasar silkworm. On these host plants, *T. fletcheri* is considered as serious pest that causes about 40-50% foliage loss during its peak period of incidence (Thangavelu and Singh 1991). Apart from direct damage it also causes the stress to plant indirectly by reducing in photosynthesis rate, respiration rate and stomatal conductance (Kar *et al.*, 2013).

Life cycle:

Trioza fletcheri is a hemimetabolous insect with 3 developmental stages *viz.*, egg, nymph and adult (Fig.1) The female generally lays 200-500 eggs singly on the leaf surface of tender and newly sprouted shoots. The eggs are slightly yellowish in colour at the time of oviposition and within 12 hours it turns to black. Further eggs will hatch after 3 days of oviposition and newly hatched nymphs starts feeding on plant sap and it continues its development and passes through five nymphal stages. The final instar nymph after completing its development emerge from the enclosed gall through a slit like opening within 20-22 days of its entrance. It then takes some rest on the leaf surface and after shedding the final nymphal skin on the leaf, fly away as adult and the life cycle thus continues with 6-7 generations a year.

Period of occurrence:

The psyllid appears during the month March on fresh leaves of *T. arjuna* and *T. tomentosa*. Their population remains till last week of December or until the beginning of leaf fall, however, the peak infestation period is from July to September.

Nature of damage:

The insect induces galls by secreting a chemical substance called cecidogen which activates perturbation and also alters the differentiation processes in the host plants, modifying the plant architecture to its advantage. Ultimately infested leaves exhibits presence of gall like structures on leaf surface and these galls are initially greenish but later on turns to brownish or yellow gland like structure, looking like small pox on the surface of leaf (Fig. 2).

Integrated Pest Management of *Trioza fletcheri minor*:

Cultural

- Two times deep ploughing after silkworm rearing during November – December and February – March
- Delay in pruning by a month i. e. last week of April to minimize the gall infestation

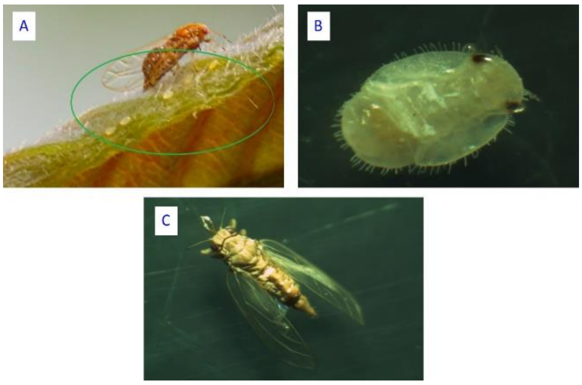

Fig. 1. Developmental stages of gall fly *Trioza fletcheri* A) Eggs B) Nymph C) Adult

Mechanical

- Collection and destruction of gall infested leaves and pruned twigs
- Installation of sticky traps during peak adult activity (July-August).

Fig. 2. Leaves showing gall fly damage symptoms

Biological

- Conservation of natural enemies such as *Trechinites aligharihnesis* (First record on association between *Trechinites aligharihnesis* and *Trioza fletcheri* reported by Thirupam Reddy *et al.*, 2021) and *Aprostocetus arjunae* (Kumar and Kumar, 2020) by avoiding the spraying of synthetic chemicals at their peak activity (Aug-Sep) would greatly help to reduce the incidence of gall fly on tasar food plants.

Botanical/Chemical

- Soil application of neem cake @ 150 kg per hectare in two split doses before the onset of monsoon (15th May and 30th May)
- Three Consecutive foliar sprays of azadirachtin (Vanguard 1500 ppm) at an interval of 15 days after sprouting of leaves (10ml/lit) with a waiting period of 7 days
- Spray Acetamiprid 20 SP@ 0.2 g/liter of water thrice with a interval of 15 days

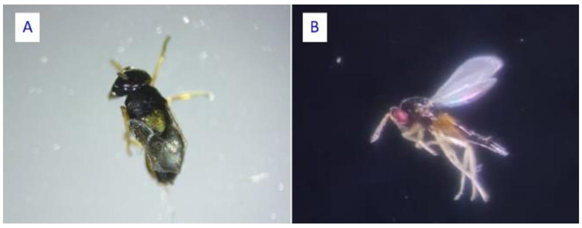

Fig. 3. Parasitoids of *Trioza fletcheri*; A. *Trechinites aligharnsis* (Hayat, Alam and Agarwal), B. *Aprstocetus arjunae* (Kumar & Kumar)

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SHORT NEWS

A conspicuous incident, *Macrocheles* mite (Acari) attached to the abdomen of *Hydrotaea* (Diptera, Muscidae)

Mites or Acari are tiny, small arachnid, cosmopolitans in nature, found worldwide. They are good decomposers, predators and some acts as a parasite on many animals and plants. Formerly Macrocheles mites were reported as predators of many dipterans fly, as they feed 1st instar larvae and eggs of the fly. During field survey at Paschim Bardhaman in West Bengal, India, we collected many *Hydrotaea sp*. (formerly synonymized as *Ophyra*) flies species under Family Muscidae (Diptera), among them we found one *Hydrotaea* fly with two *Macrocheles* sp. (acari) on the ventral surface of abdomen. *Macrocheles* (Family- Macrochelidae) acari are parasitic, fast moving mites. They show a special type of dispersal behavior known as Phoresy, uses the host by direct contact for travelling the new habitats.

The infestation of *Macrocheles* mite on the body of *Hydrotaea* fly is an important event as it poses some threats to *Hydrotaea* and other fly families also.

Macrocheles sp. (Acari) attached in the abdomen of the *Hydrotaea* sp. (Muscid fly)

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Predation of drain fly *Lispe orientalis* (Muscidae) over moth fly *Clogmia albipunctata* (Psychodidae) larvae

Drain fly *Lispe orientalis* Wiedemann 1824, is a predaceous fly belonging to family Muscidae, Order Diptera. This fly is found in almost all the Oriental countries like India, Sri Lanka, Sumatra, Malaysia and Myanmar. They are also reported from various countries of palearctic region. Commonly *Lispe orientalis* is available near the margin of waterside of pond, lake, drainages, tube-wells, wetlands where moth fly (*Clogmia albipunctata*) also breeds. Adult flies of genus *Lispe* are known as predator on small flies like Dolichopodidae, Ephydridae, Muscidae and Simulidae. But there was no published report about their predation over larvae of moth fly (family Psychodidae) which are involved to urinary and vaginal myiasis in human.

During the field survey on muscid flies near a wetland the predation behaviour of *Lispe orientalis* was observed. This is the first case report, that they actively hunt the larvae of myiasis causing moth fly (*Clogmia albipunctata*). Thus, adult *Lispe orientalis* may be considered as brilliant bio-control agent on moth fly (*Clogmia albipunctata*).

Lispe orientalis preying the larvae of moth fly *Clogmia albipunctata*

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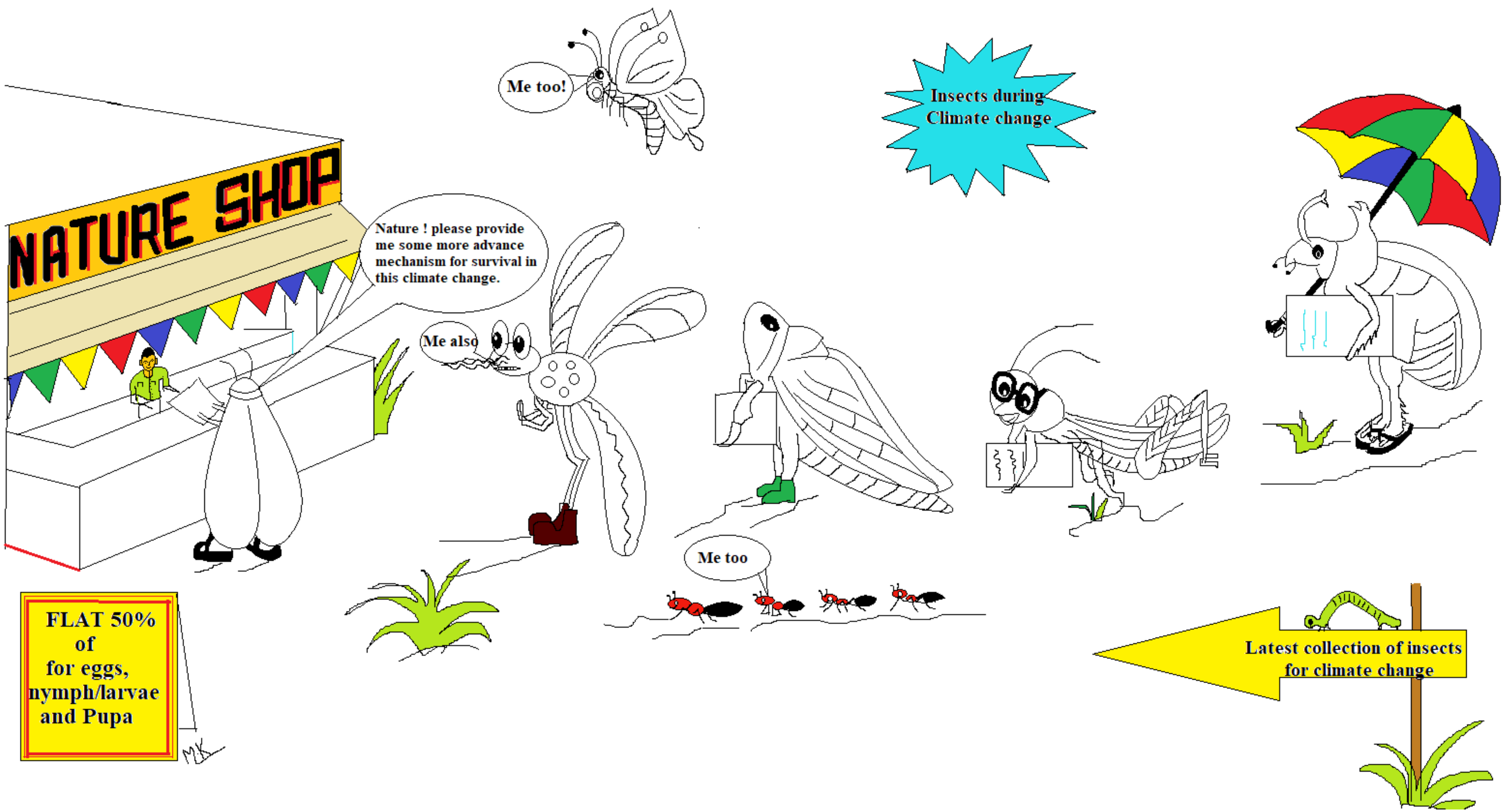

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SIXTH INDIAN ENTOMOLOGIST PHOTO CONTEST

The Indian Entomologist photo contest aims to encourage insect photography among photographers, professionals, amateur entomologist and the layman. The theme of the sixth episode of the photo contest was ‘Insects and aspects related to insect life’. With these objectives, entries were invited during 5th May to 20th June 2022. Each participant was to submit one good photograph which met a few prescribed standards along with the filled in application form in which the participant had to furnish his/ her details, caption, description, specifications of the photograph and also a declaration on the ingenuity of the photograph.

In the Sixth photo contest, we received less entries which is quite a low number compared to the previous contests. These were screened first for the prescribed standards and overall quality of the image. Final evaluation was done by a committee of independent members under the oversight of the three editorial board members and also by an invited expert, based on the following criteria: quality (clarity, lighting, depth of field, composition), relevance of the subject matter (theme, rareness of subjects), creativity and originality. To ensure a blind review the details of the photographer was hidden and the evaluators were only presented with the photograph, caption, description and technical specifications. Since the number of entries were low we decided to select a **best photo.**

- The best photo award was given to Dr Santhoshkumar C Kedar (Division of Crop Production and Protection, CSIR - Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow - 226015, Uttar Pradesh, India E-mail: santoshkedar@cimap.res.in) who captured the emergence of Cicada (*Chremistica* sp.).

BUG STUDIO ASSOCIATE EDITORS

Best Photo: *Cicada emerging (Chremistica* sp.). Photo by Dr. Santhoshkumar C Kedar (Division of Crop Production and Protection, CSIR - Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow - 226015, Uttar Pradesh, India. Photo taken on 01.07.2018, Apple Iphone X with, ISO-32, 35 mm focal length 28.0 mm with flash auto, from Jiji Town, Nantou County, Tiwan.

MANGALI ASHWINI

DEPARTMENT OF ENTOMOLOGY, NAVSARI AGRICULTURAL UNIVERSITY, NAVSARI, GUJARAT

Mangali Ashwini is pursuing her Ph. D. from Department of Entomology, Navsari Agriculture University, Navsari, Gujarat. She is currently working on the biodiversity of Coccinellids in South Gujarat under the guidance of Dr. Abhishek Shukla (Professor & Head). Her focus of interest is to record the diversity of Coccinellids in different ecosystems along with the associated host plants and prey insects. She is also working on DNA barcoding and trying to establish evolutionary relationship among the Coccinellids. In addition, she is studying the biology of some of the dominant coccinellid species. She believes that nature, with a huge treasure of biodiversity, never fails to surprise us. It is always important to explore the natural enemies which can bring out new possibilities for effective control of insect pests. In future, she intends to carryout biosystematic studies in other insect groups along with molecular taxonomy.

KARTHIK REDDY M

NATIONAL PUSA COLLECTION, DIVISION OF ENTOMOLOGY, ICAR-INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI

Karthik Reddy M is currently pursuing his M. Sc. from the Division of Entomology, ICAR-Indian Agricultural Research Institute, Pusa, New Delhi. He is working on Biosystematics of subfamily Olethreutinae (Lepidoptera: Tortricidae) under the supervision of Dr. Shashank P R, Scientist, ICAR-IARI, Pusa, New Delhi. Tortricids are associated with various plants as stem-borers, leaf-miners, fruit-borers, seed-borers and gall-inducers, and some are considered as agricultural pests of international quarantine importance. Contrastingly, many olethreutine moth species are being continuously assessed for their role as biocontrol agents of weed species around the world. He has studied the olethreutine fauna of Southern Karnataka, giving emphasis to classical taxonomic characters and developed illustrated diagnostic keys for their easy identification. He has also generated DNA barcodes for some of the important agricultural pests and weed control agents of the subfamily Olethreutinae. He is interested to continue his future research in Lepidopterology focusing on the taxonomy, biology and host-association of economically important families.

STUDENT CORNER

SASHANKA SEKHAR DASH

DEPARTMENT OF ENTOMOLOGY, COLLEGE OF AGRICULTURE, OUAT, BHUBANESWAR

Sashanka Sekhar Dash is pursuing his Ph. D. from the Department of Entomology, College of Agriculture, OUAT, Bhubaneswar, Odisha. He is currently working on Spatio-temporal changes in toxicity to different insecticides and enzymatic resilience factor in mango hopper under the guidance of Dr. Manoj Kumar Tripathy (Professor, Department of Entomology, College of Agriculture, OUAT, Bhubaneswar). Mango hopper is one of the most important sucking pests among the pest complex of mango in Odisha condition. To evaluate the efficacy of certain chemicals against mango hopper he is doing the LC_{50} study over three different places in Odisha. Along with this he is also quantifying and studying the changes in the titers of detoxifying and protective enzymes present in mango hopper, after the insecticide spray. In future, he is interested to extend his work on toxicology, which is an important asset in pest management.

NEETHU ROY

DEPARTMENT OF ENTOMOLOGY, UNIVERSITY OF AGRICULTURAL SCIENCES, RAICHUR, KARNATAKA

Neethu Roy is pursuing her Ph. D. in Agricultural Entomology from University of Agricultural Sciences, Raichur, Karnataka. Under the guidance of Dr. Arun Kumar Hosamani (Professor and Head, AICRP on Biological Control, Main Agriculture Research Station, UAS, Raichur, Karnataka), she is currently working on efficacy of the entomopathogenic fungus, *Metarhizium anisopliae* (Metchinkoff) Sorokin against tobacco caterpillar, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *S. litura* is a major and polyphagous pest infesting various crops in India. In order to overcome the negative effects of pesticides that are being targeted for this pest, she is working on native isolates of the naturally occurring fungi, *M. anisopliae* for controlling *S. litura* and also extracting a toxin from it, called destruxin. She is microencapsulating and gel formulating the fungal spores targeting to control *S. litura*. Besides, she is also studying the cuticle degrading enzymes of *M. anisopliae* which helps in degrading the cuticle of the host insect for attaining successful entry of the fungus. In future, she hopes to work on biological pest control using other species of insects (parasitoids and predators) and pathogens that will help to control insect pests in conjunction with other tools of pest management.

Mr. Kishore Chandra Sahoo, Ms. Akshatha, Mr. Sanath R M, Student Associate Editors of IE compiled the information for this section.

Indian Entomologist is a biannual on-line magazine and blog site that publishes articles and information of general, scientific and popular interest. The magazine publishes letters to the editor, columns, feature articles, research, reviews, student opinions and obituaries. The magazine accepts articles on all aspects of insects and terrestrial arthropods from India and worldwide. Short field notes and observations are also welcome. This magazine is intended to provide a broad view of topics that appeal to entomologists, other researchers interested in insect science, and insect enthusiasts of all stripes.

Notes for Contributors

Articles submitted should not have been published elsewhere and should not be currently under consideration by another journal/ magazine. Interested authors are advised to follow the author guidelines of Indian Journal of Entomology for reference citations and to follow as closely as possible the layout and style, capitalization and labelling of figures. All papers are subject to peer review and may be returned to the author for modification as a result of reviewers reports. Manuscripts are acknowledged on receipt and if acceptable proofs are sent without further communication. Minor editorial alterations may be made without consulting the author. Make sure to submit the photographs of high quality in .jpg format. For those who want to contribute commentary and feature articles please contact editors before submission.

About articles

IE is intended to publish following categories of articles

Commentary – We encourage opinions or critical analysis of current entomological happenings. Submissions should be no more than 5,000 words in length.

Reviews – two types of reviews will be published a. invited review (editorial team will contact eminent entomologists to contribute) and b. peer reviewed review (any author/s can submit a comprehensive reviews on modern entomological developments).

Feature articles – these must be of broad interest to biologists, amateur and professional entomologists. These articles should be no longer than approximately 5,000 words. Articles should contain high quality photographs.

Natural histories & short research articles- with focus on insect life cycle, occurrence etc. and have the same requirements as feature articles. Submissions should be up to 5,000 words in length.

Field notes - on unusual observations entomologists encounter during fieldwork (Invasive insects, outbreaks, behaviour etc.). Submissions should be no more than 2,000 words in length.

Bug studio- "Indian Entomologist Photo Contest" will be conducted for every volume of the magazine and best three winners will be announced in the magazine. Images should be submitted as high quality (300 dpi TIFF, jpeg files) files with a detailed photo caption. The announcement for photo contest will be made on our website www.indianentomologist.org

Student corner- students working on interesting topics of entomology to share their views and opinions about their research work. Can submit with personal photograph; it should not be more than 1,000 words in length.

We encourage entomologists to contact us if you have any interesting story to share about insects.

Contributions to be sent to the Managing Editor, in digital format (MS Word) as an e-mail attachment to indianentomologist@gmail.com

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