Population and genetic diversity in banana pseudostem weevil and leaf and fruit scarring beetle, inferred from RAPD fingerprints

A Dissertation submitted in partial fulfilment of the requirements for the Master of Philosophy

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Submitted

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CERTIFICATE

I certify that the dissertation entitled "**Population and genetic diversity in banana pseudostem weevil and leaf and fruit scarring beetle, inferred from RAPD fingerprints**" submitted to Mizoram University for the award of the degree of Master in Philosophy in Zoology by **Abinash Giri** a record of research work carried out during the period of 2019 under my guidance and supervision, and that this work has not formed the basis for the award of any degree, diploma, associateship, fellowship or other titles in this university or any other university or institution of higher learning.

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I, **Abinash Giri**, hereby declare that the subject matter of this dissertation entitled " **Population and genetic diversity in banana pseudostem weevil and leaf and fruit scarring beetle, inferred from RAPD fingerprints**" is the record of work done by me, that the contents of this dissertation did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the dissertation has not been submitted by me for any research degree in any other university/Institute.

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4.	**Topic of Research**	:	**Population and genetic diversity in banana pseudostem weevil, *Odoiporus longicollis* Oliver (Coleoptera: Curculionidae) inferred from RAPD fingerprints**
5.	**Supervisor**	:	**Prof. G. Gurusubramanian**

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6. Abinash Giri, concerned Scholar, Deptt. of Zoology, MZU.

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Abinash Giri

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CHAPTER 1

INTRODUCTION

1.1 Edible and wild banana cultivars from Mizoram

Banana is deliberated as one of the vital food items by means of it is high in nutrients and profitable value all over the tropical world. It is considered as a significant tropical natural product crop disseminated in 120 nations with a yearly production of 120 million tonne (FAO, 2005). Banana has a place with the family *Musaceae*, *Zingiberales* order, which comprises of two genera *Musa* L. and *Ensete* Bruce. It is profoundly expanded all through the world however answered to have begun from Southeast Asia (Simmonds, 1966). The genus *Musa* comprises of about 50 species whereas *Ensete* has 9 species (Azhar and Heslop-Harrison, 2008; Simmonds and Shepherd, 1955). Based on phenotypic qualities and essential chromosome number, *Musa* has been separated into four areas specifically, *Eumusa*, *Rhodochlamys*, *Austra-limusa* and *Callimusa* (Simmonds and Shepherd, 1955). The *Eumusa* comprises the wellsprings of eatable bananas got from the two wild diploid ancestor species *M. acuminata* (assigned as AA genome) and *M. balbisiana* (assigned as BB genome) which prompts the root of various ploidy levels of banana assortments (AB, AAB, ABB and ABBB) through intra and between explicit crosses (Uma *et al*., 2006). India is the biggest maker with the yearly creation of 13.5 MT from a territory of 4 lakhs ha (Sathiamoorthy *et al*., 2001).

Northeast India has been considered as the richest sources of banana diversity in which the clones of *M. balbisiana* from Indian subcontinent hybridize with *M. acuminata* from Southeast Asia (Molina and Kudagamage, 2002). North-east India is considered as the reservoir for the large gene pool of banana genetic resources, and is the meeting point of *Musa balbisiana* of the Indian subcontinent and *Musa acuminata* of Southeast Asia. With the loss of crop genetic resources at an alarming rate, the future of global food crops depend on the sustainability of the genetic pool at their centre of diversity. The North-eastern states of India, namely Assam, Arunachal Pradesh, Meghalaya, Tripura, Mizoram and Manipur have been richest sources of natural diversity. Altogether 39 different accessions of banana have been collected and characterised. From the state of Mizoram, 14 different accessions have been collected and characterized (**Tables 1** and **2**). The important commercial banana

varieties of the state are vaibalhla (*M. acuminata* AAA group), lawngbalhla (Musa AAB group) and banria (*Musa* ABB group) (Lalrinfela and Thangjam, 2012).

Table 1. List of edible banana found in Mizoram.

Scientific Name	Edible banana	Genome Type	Morphological score (IPGRI, 1996)
Musa paradisiaca	Banria	ABB	**62**
	Balhlasen	AAB	**38**
	Banthur	AAB	**39**
	Banpawl	AB	**59**
	Lawngbalhla	AAB	**33**
	Kawlbalhla	ABBB	**66**
	Vaibalhla	AABB	**60**
	Khumtungbalhla	AAB	**50**
	Zobalhla	AAB	**48**
	Changkha	AABB	**46**
	Kawrmuat	AAAB	**35**

(**Uma *et al.*, 2001**)

Table 2. List of wild banana cultivars found in Mizoram.

Wild banana	Scientific Name	Genome Type	Morphological score (IPGRI, 1996)
Changthir	*Musa balbisiana*	BB	**70**
Changpui	*Musa acuminata*	AB	**46**
Changpawl	*Musa paradisiaca*	ABBB	**50**
Changvandawt	*Musa ornata*	--	**-**
Lairawk	*Musa paradisiaca*	AB	**46**
Lairoop	*Musa paradisiaca*	AB	**47**
Saisu	*Ensete glaucum*	--	--

(Uma *et al.*, 2001)

1.2 Banana pests - *Odoiporus longicollis* and *Basilepta subcostatum*

Banana is attacked by number of pests, among them banana pseudostem weevil *Odoiporus longicollis* (Oliver) and leaf- and fruit- scarring beetle *Basilepta subcostata* (Jacoby) are major monophagous pests which limiting the production and productivity, posing serious threat to banana production (Visalakshi *et al.*, 1989; Valmayor *et al.*, 1994; Shukla and Kumar, 1970; Prathapan *et al.*, 2019). *O.*

longicollis causes damage that ranges from 10 % to nearly 90 % subject upon the phase of plant growth at which pest infestation occurs and the yield of the management practices that are being followed (Padmanaban and Sathiamoorthy, 2001). In addition to these, leaf- and fruit-scarring beetles (Coleoptera, Chrysomelidae) are key periodic pests of bananas and plantains in many states of northern, eastern, and north-eastern India, Bangladesh, and parts of Southeast Asia (Prathapan *et al.*, 2019).

1.3 Population dynamics of *Odoiporus longicollis* and *Basilepta subcostatum*

The study of population and occurrence of *O. longicollis* is very much important for understanding the status and rate of population according to the season and also to establish a well-developed control measures of this pest (Azam *et al.*, 2010). *O. longicollis* is popularly known as an internal feeder pest of banana crop. All life stages of *O. longicollis* remains active for throughout the year. There is no hibernation period for them. The adult male and female weevils can reproduce in both summer and winter seasons (Devi *et al.,* 2015). They are present inside the pseudostem for all seasons but depending on the season, the rate of population varies. The activity of *O. longicollis* enhances during July to September i.e. in the monsoon period and gradually slows down from November to January (Priyadarshini *et al.*, 2015). Based on the population rate of *O. longicollis* the rate of infestation can be studied (Thippaiah *et al.,* 2010). The initial incidence of *O. longicollis* generally found from six month old plantation and with the growing stages of the banana crop the population of *O. longicollis* also increases gradually. The population rate of *O. longicollis* also very much dependent on the meteorological parameters such as maximum temperature, minimum temperature, relative humidity, rainfall (Biswas *et al.,* 2015). The population of the *B. subcostatum* are too high during the rainy seasons i.e. from April to September. Winter is the season of hibernation for the adult beetles (Sharma and Saikia, 1967).

1.4 Life cycle of *Odoiporus longicollis*

The life cycle of the *O. longicollis* from egg to the adult were seen in banana pseudostem in summer and winter season (Padmanaban and Sathiamoorthy, 2001). *O. longicollis* infested cultivars can be recognized so easily by the help of small holes made by the weevils while feeding upon the pseudostem. These holes are found in the pseudostem of the cultivar. The elliptical, yellowish white eggs are laid by the female adult weevil under the outer leaf sheath of pseudostem. During the complete life cycle of the banana pseudostem weevil the larvae pass through 4^{th} instar in the developmental stage. The apodous larvae are fleshy and yellowish white in colour. After the completion of 4^{th} instar stage of the larvae they stops feeding and starts resting for pupa stage of their life inside self-made cocoon. Pupa develops into adult or the last life stage of the weevil. The adults are generally 20-30 mm long in size. In India both the black and red coloured adults are available (Priyadarshini *et al*., 2014). These colour differences in adult weevils are not because of the sexual dimorphism. According to the mating studies it is the result of the phenomenon of non-sex limited variation and of sympatry (Dutt and Maiti, 1972).

1.5 Life cycle of *Basilepta subcostatum*

The egg is oval in shape and pale lemon yellow in colour. The larva is white with dark coloured head. During the complete life cycle of scarring beetles they passed their maximum life stages in the soil. They used to lay eggs in the soil and pupation also occurs in the soil, only the adults are found inside the leaf whorl. As they are known as night loving insects, during day time they hide themselves inside the curled leaf until any interference by anyone. The population of the scarring beetle are too high during the rainy seasons i.e. from April to September (Sharma and Saikia, 1967). Winter is the season of hibernation for the adult beetles.

1.6 Population genetic structure analysis - Random Amplified Polymorphic DNA

For the study of genetic diversity in different organisms Random Amplified Polymorphic DNA (RAPD) fingerprinting have been used which is a PCR-based

technique. The amplification products produced by RAPD primers anneal to homologous target sites of the template DNA, in which the genomes are randomly distributed (Williams *et al*., 1990; Welsh and McClelland, 1990). RAPDs are delicate enough to notice dissimilarities between individuals showing a close genetic relationship. The key benefit of this method is that it can be useful with few necessities for modelling, assumptions or analysis. Besides, this technique has been confirmed to be useful in exposing geographical origins and scattering routes of insect pest populations, mostly *Curculionidae* weevils (Taberner *et al.*, 1997; Bas *et al*., 2000; Scataglini *et al*., 2000; Kim and Sappington, 2004).

To study the population of numerous insects, mitochondrial genes have been used because they have numerous significant characteristics i.e. those are haploid, inherited from maternal, absence of introns, progress more quickly than nuclear coding genes, deficiency of recombination and are expected to differ in a neutral manner. Comparing 12S and 16S rDNA genes the mitochondrial protein coding region rate of evolution is much faster and hence those regions help as useful markers for reading evolutionary history at the periods of family, genera and species (Wan *et al*., 2004). Interpreting phylogeny and phylogeography mitochondrial genes have been used in several insects (Orsini *et al*., 2007) including *Heliconius butterflies* (Brown, 1994), *Halys fabriciusm* (Memon *et al*., 2006), *Diabrotica* (Szalanski *et al*., 2000), *Adelges cooleyi* (Ahern *et al*., 2009), *Aphidus ervi* (Hufbauer *et al*., 2004) and *Apis cerana indica* F (Baskaran, 2011).

In limited studies, mitochondrial genes have not shown useful for the assessment of phylogeography at the intraspecies level. For example, intraspecific phylogeography of *Apis cerana* did not associate with geographic distribution when COI/COII area was used for association of dissimilar geographic populations (Hepburn *et al*., 2001). In *Tomincus destruens*, Woll Horn *et al*, (2006) gained no clear phylogeographic pattern within geographic populations.

1.7 Population genetic structure analysis - Mitochondrial Cytochrome Oxidase I Marker

Compared to nuclear markers, mitochondrial markers are more susceptible to the effects of genetic drift (Filipova *et al*., 2011). As a powerful and widely used molecular marker, mtDNA has been applied in many organisms to determine the genetic variations and structure of population (Xu *et al*., 2011). Mitochondrial DNA has become a major tool of comparative genomics and occupies a significant role in genetic structure of population and molecular variations as it is maternally inherited with no intermolecular genetic recombination with rapid rate of evolution (Near *et al*., 2003; Cardenas *et al*., 2009; Xu *et al*., 2011). COI is a protein-coding gene in mtDNA. Due to fast evolution, high polymorphism, easy amplification and sequencing, it has shown valuable information and is a widely used genetic marker for population genetic studies especially intra-specific analysis (Near *et al*., 2003; Hu *et al*., 2008; Cardenas *et al*., 2009; Xu *et al*., 2011).

Mitochondrial markers are more sensitive compared to nuclear markers for the effects of genetic drift (Filipova *et al*., 2011). mtDNA has been working as an influential and extensive molecular marker in many organisms to govern the genetic variations and structure of population (Xu *et al*., 2011). Mitochondrial DNA has converted to a major tool of relative genomics and occupies an important role in genetic structure of population and molecular dissimilarities as it is inherited from maternally with no intermolecular genetic recombination with rapid rate of evolution (Near *et al*., 2003; Cardenas *et al*., 2009; Xu *et al*., 2011). COI is a broadly used genetic marker because it is a protein-coding gene of mitochondria having rapid evolution, high polymorphism and easy amplification and sequencing (Near *et al*., 2003; Hu *et al*., 2008; Cardenas *et al*., 2009; Xu *et al*., 2011).

CHAPTER 2

REVIEW OF LITERATURE

2.1 Edible and wild banana cultivars from Mizoram

The economic review for the identification of the potential banana cultivars inborn to Mizoram was identified as Vaibalhla (AAA), Banria (ABB) and Lawngbalhla (AAB). The most popular cultivar with the maximum economic value was Vaibalhla. The cultivar of Vaibalhla is a triploid *M. acuminata* (AAA) of the Cavendish sub-group having a sweet taste (Lalrinfela and Thangjam, 2012). It has been described by Lescot (2011) that half of the current banana production depend on somaclones derived Cavendish dessert bananas (AAA group). The most vital qualities that make the Cavendish sub-group the main bananas for transfer are related to their consistency during transport and their shelf life. Between the 3 cultivars identified, 2 (Banria and Lawngbalhla) were found throughout the state while Vaibalhla was found only in the tropics (Hrahsel and Thangjam, 2013). It is a clear known point that banana (*M. acuminata*) having the A genome are more cold-sensitive as associated to plantains (*M. balbisiana*) having the B genome, even though the biological mechanisms of cold-tolerance for plantains are still not clear (Zhang *et. al*., 2011). The Vaibalhla (AAA) was not found in the temperate region of Mizoram (Champhai district), while Banria (ABB) and Lawngbalhla (AAB) flourishes well in all the phytogeographical regions including the temperate regions (Hrahsel and Thangjam 2013).

2.2 Biology and Population dynamics of *Odoiporus longicollis*

An widespread record on the biology and pest status of the banana pseudostem weevil, *O. longicollis*, was recorded by Dutt and Maiti (1972), in this way Isahaque (1978), Shukla and Tripathi (1978), Visalakshi *et al*, (1989), Padmanabhan and sathiamoorthy (2001), Tiwari *et al*, (2006) and Thippaiah *et al*, (2010), furthermore exposed the biology of *O. longicollis* all in all. Azam *et al*, (2010) by their examination to discover the event, mode, degree of harm, life design of pseudostem weevil of banana filled in Poonch and Rajouri regions of Jammu and the vermin population during several seasons and field conditions it has been suggested that to strategy for a practical control measure, information on the frequency of the impatience and the number of populaces in the weevil during various seasons is a lot

of important. Priyadarsini *et al*, (2014) considered about bioecology and seasonal incidence pattern *O. longicollis* in cooperation with field and laboratory conditions. Krishnan and Jayaprakas (2015) intense on the bionomics, circulation and the executives of the banana pseudostem weevil and determine that impulsive utilization of compound assistances to farming poses dare to practical agriculture is possible simply by considerate the pests in its morphological, taxonomical, natural and distribution levels. The way of occurrence of *O. longicollis* was studied by field inquiry under gangetic tract of West Bengal (Biswas *et al*., 2015). Devi *et al*, (2015) stated that *O. longicollis* are found active in all seasons and maximum in the month of September through their study on the population structure and seasonal incidence of the pest.

The leaf sheaths have spaces where mating occurs and in the one air chamber present inside the leaf sheath they laid one egg. The shape of egg is cylindrical and yellowish white in colour. Larvae consist of five larval instars and they are apodous, soft having dark brown head. Extended cylindrical cocoons are made for pupation by twisting short pieces of chewy materials of the leaf sheath. The adults were Black and reddish brown noted from disassociated pseudostem of infested banana cultivars. The weevil breeds all over the year and do not go through winter rest (Azam *et al*., 2010).

Incongruencies detected in the phylogenies constructed on mitochondrial and nuclear genes have completed studies found on both groups of genes significant in insect molecular systematics (Shankar *et al*., 2015). Supervision of *O. longicollis* is a criterion to satisfying productivity and to obtaining higher economic profits of bananas and plantains. A serious aspect for evolving a successful integrated pest management (IPM) policy for the regulator of this pest is the study of the population structure of the pest, i.e. measuring the genomic variability of the pest among and within sites and how this variability is separated geographically. The internal transcribed spacers of I and II of rDNA have been used to measure the genetic diversity of *O. longicollis* entities composed from six hotspot locations in India (Kumar *et al*., 2018).

The larvae and pupae those live in inside the pseudostem can endure the storage circumstances during transport. Biological structures increase the chance of establishment of these weevils in fresher areas (Kumar *et al*., 2018). 2-Methyl-4-heptanol (2M4H) was testified as the male-secreted combination pheromone of *O. longicollis* (Gunawardena *et al*., 1997). Male weevils were reactive to male as well as female extracts whereas female receptive towards only male extract (Prasuna *et al*., 2008). The pests breed throughout the year and do not undergo dormancy (Azam *et al*., 2010).

Williams *et al*., (1991) defined a technique that they called RAPD (Random Amplified Polymorphic DNA) in which a ten oligonucleotide primers of random sequence but with a least of 50% guanine-cytosine contents. The polymerase chain reaction (PCR) is a highly effective technique of amplifying distinct DNA fragments using a thermo stable DNA polymerase with single-stranded DNA primers. An application of the PCR technique that uses DNA primers of arbitrary nucleotide sequence to amplify arbitrary regions of the genome has been described (Welsh *et al*., 1991; Williams *et al*., 1991).

2.3 Biology and population dynamics *Basilepta subcostatum*

An investigation was considered in the natural plantation of banana to scrutinise the population structure of leaf and fruit scarring beetles, *B. subcostatum* found in Assam Agricultural University, Jorhat (Mishra *et al*., 2015). To know the periodical occurrence of *B. subcostatum* on banana plantation Sah *et al*, (2018) considered on the population build up and invasion of *B. subcostatum*. By this experiment they found that the population construction of this pest in dependent on temperature and at low temperature the population rate goes down and as well as infestation rate also. To determine the structural composition of different species of leaf and fruit scarring beetle in the northern and Northeastern regions of India, Prathapan *et al*, (2019) studied on species configuration of this pest through taxonomy and COI sequence analysis.

2.4 Population genetic structure analysis by Random Amplified Polymorphic DNA

Genetic distance matrices generated by RAPDs are low level of connection RAPDs and revealed polymorphisms in the coding as well as in the non-coding regions and can possibly cover the whole genome (Shankar *et al*., 2014). The UPGMA dendrogram resulting from RAPDs clusters in the individuals rendering to the sampling locations and AMOVA analysis displays that nearly half of the observed genetic variation happens within the populations and this indicate that the banana rhizome weevil had formed local populations due to limited dispersal (Yadav *et al*., 2017).

2.5 Population genetic structure analysis - Mitochondrial Cytochrome Oxidase I Marker

The gene mutation inherited from maternal mitochondrial genome was frequently derived from different sequence. The study of intraspecific polymorphism of COI is valuable information derived from mitochondrial genome (Barbaresi *et al*., 2003). The COI sequence provides well Understanding of characteristics of genetic structure population (Liu *et al*., 2013).

The L2 gene of insects and crustaceans, encoding the two codon families of UUR, lies among the COI and COII genes. The COI-tRNA^{Leu} -COII sequenced region displayed the distinctive AT bias as observed in insect mtDNA (Frati *et al*, 1997). The GA and CT (U) transitions were extra frequent than the T(U)C and AG transitions (Shankar *et al*., 2014).

Though *O. longicollis* is a serious pest of banana, there are no sufficient reports on the characterisation of this pest using molecular markers in Northeast India.

CHAPTER 3

OBJECTIVES

- Biology, seasonal abundance, population dynamics and host preference of banana pseudostem weevil, *Odoiporus longicollis* and banana scarring beetle, *Basilepta subcostatum* in banana growing regions of Mizoram.

- Host- based genetic differentiation of *O. longicollis* by using RAPD markers.

- Verification of mitochondrial COI markers to recognize phylogeographical relationships among *O. longicollis* and *Basilepta subcostatum* to reveal their population genetic structure.

CHAPTER 4

MATERIALS AND METHODS

4.1 Sampling and collection

O. longicollis and *B. subcostatum* were collected from the banana cultivars from the four districts of Aizawl, Lunglei, Lawngtlai and Saiha (**Table 3**). Specimens from different populations were collected by directly under the leaf sheath of recently infested trees. Collected beetles were kept in 70% ethanol in -20 ºC (Yadav *et al*., 2017).

4.2 Identification and characterization of Banana cultivars in Mizoram

The taxonomical classification and identification of the collected banana cultivar samples were carried out by assessing the habit, leaf, floral and fruit features using the identification keys provided by Singh *et al*, (2012) and Häkkinen, (2013). For genome classification, the morphological characters of vegetative, male and female inflorescence based on 15 characters suggested by Simmonds and Shepherd, (1955) were evaluated (**Table 4**) and a relative score was recorded (Uma *et al*., 2001; IPGRI, 1996). For example, with respect to pseudostem colour, score of 1 is given, if the pseudostem is heavily blotch with brown or black pigmentation. Similarly, a maximum score of 5 was given when blotches are completely absent and the pseudostem is more or less green. Intermediary scores from 1-5 depending on the extent of blotching and the score range from 1-75 (**Table 4**).

Table 3 List of geographical population.

Sl No	Location	Coordinates	Districts	Banana Variety	*Musa* sp.
1	Tanhril 1	23.737, 92.663	Aizawl	Changthir	*balbisiana*
2	Tanhril 2	23.734, 92.668		Balhlasen	*paradisiaca*
3	Tanhril 3	23.737, 92.670		Banria	*paradisiaca*
4	Tanhril 4	23.737, 92.701		Vaibalhla	*paradisiaca*
5	Tuirial	23.759,92.635		Changpawl	*paradisiaca*
6	Sakawrtuchhum	23.759, 92.651		Changpui	*acuminata*
7	Tanhril 5	23.737, 92.663		Vaibalhla	*paradisiaca*
8	Tanhril 6	23.737, 92.663		Banria	*paradisiaca*
9	Tanhril 7	23.737, 92.663		Banria	*paradisiaca*
10	Theriat	22.735, 92.471	Lunglei	Zoblhla	*paradisiaca*
11	Theriat	22.731, 92.465		Khumtungbalhla	*paradisiaca*
12	Theriat	22.731, 92.465		Khumtungbalhla	*paradisiaca*
13	Tuipui	22.879, 92.935		Khumtungbalhla	*paradisiaca*
14	Darzo	22.833, 92.955		Khumtungbalhla	*paradisiaca*
15	Vanlaiphal	22.803, 92.995		Vaibalhla	*paradisiaca*
16	Sangau	22. 441, 93.410	Lawngtlai	Khumtungbalhla	*paradisiaca*
17	Cheural	22.707, 93.015		Vaibalhla	*paradisiaca*
18	Cheural	22.707, 93.015		Khumtungbalhla	*paradisiaca*
19	Rawlbuk	22.673, 92.996		Vaibalhla	*paradisiaca*
20	Saiha	22.489, 92.979	Saiha	Vaibalhla	*paradisiaca*

Table 4. Morphological characters used for banana classification (Simmonds and Shepherd, 1955).

Sl No	Characters	*Musa acuminata*	*Musa balbisiana*
1	Pseudostem colour	More or less heavily marked with brown or black blotches	Blotches slight or absent
2	Petiolar canal	Margin erect or spreading, with scarious wings below, not clasping pseudostem	Margin enclosed, not winged below, clasping pseudostem
3	Peduncle	Usually downy or hairy	Glabrous
4	Pedicel	Short	Long
5	Ovules	Two regular rows in each loculus	Four irregular rows in each loculus
6	Bract shoulder	Usually high (ratio < 0.28)	Usually low (ratio < 0.30)
7	Bract curling	Bract reflex and roll back after opening	Bract lift but do not roll
8	Bract shape	Lanceolate or narrowly ovate, tapering sharply from the shoulder	Broadly ovate, not tapering sharply
9	Bract apex	Acute	Obtuse
10	Bract colour	Red, dull purple or yellow outside; pink, dull purple or yellow inside	Distinctive brownish-purple outside; bright crimson inside
11	Colour fading	Fading inside bract colour fades to yellow towards the base	Inside bract colour continuous to base
12	Bract scars	Prominent	Scarcely prominent
13	Free tepal of male flower	Variably corrugated below tip	Rarely corrugated
14	Male flower colour	Creamy white	Variably flushed with pink
15	Stigma colour	Orange or rich yellow	Cream, pale yellow pale pink

4.3 Life cycle of Odoiporus longicollis and Basilepta subcostatum

Life cycle of the insect was studied in the laboratory conditions (27 ± 3 °C, 60 ± 10% RH and L:D 12:12). Mating behaviour, pre-oviposition and oviposition behaviour, egg and incubation period, larva, feeding behaviour, pupation, pupa and adults were studied. Images were taken by camera under necessary zoom. Cocoons collected from the infested plants were individually reared in 100 mL plastic cups with in emergence, one male and one female each of 13 days old was confined for mating in a 100 mL plastic container for 24 h, and was provided pseudo stem pieces of 4 x 3 cm for feeding and egg laying. In order to understand the mating frequency and fecundity, two sets of experiment was conducted; one with female exposed to male only for 24 h whereas in the other set male and females will exposed

continuously till their death. Five replications were maintained (Krishnan and Jayaprakas, 2015).

4.4 Population and infestation studies of *Odoiporus longicollis* and *Basilepta subcostatum*

The size of population and infestations studies of *O. longicollis* and *B. subcostatum* were conducted from August, 2019 to January, 2021 in four district of Mizoram. Arbitrarily four banana orchards were selected from each site. The population of *O. longicollis* and *B. subcostatum* were studied from haphazardly selected plants. The *O. longicollis* population was studied by taking account of weevils on pseudostem and holes created by the weevils (i.e. per 30 cm^2 area) from the number of holes the pseudostem was studied. For *B. subcostatum* the overall size of population was calculated the number of beetles found on leaf surface and inside the cigar. Infestation pattern was counted by the sum up the number of scars presented on per 5 cm^2 area of banana leaf surface (Mishra *et al*., 2015).

4.5 Random Amplified Polymorphic DNA fingerprints of *Odoiporus longicollis* and *Basilepta subcostatum*

4.5.1 DNA extraction

DNA was extracted by using Sambrook and Russell (2006) with some modifications. Samples were washed twice with phosphate buffered saline (PBS) (500 µL), centrifuged at 12,000 rpm for 10 min and dried. 300 µL of buffer (100 mM Tris HCl, 50 mM EDTA, 100mM NaCl, 200 mM Sucrose and 1% SDS) and TEX buffer (1 M Tris HCl, 0.5 M EDTA and 1% Tripton 100) was added respectively, to the sample placed in mortar pestle and crushed the samples. After crushing 10 µL proteinase K (50 mM Tris HCl and 10mM $Cacl_2$) was added in each tube and vortex done vigorously in each tube for 1 min and kept for overnight incubation at 55 °C in thermo cycler at 1300 rpm. The samples were kept in room temperature for 10 min centrifuged at 14,000 rpm for 10 min. Took supernatant in separate tube and added 500 µL of phenol: chloroform: isoamyl alcohol (25:24:1). The solutions were mixed by inverting for 2 min and centrifuged at 14,000 rpm for 10 min. Supernatant was taken in the fresh tubes and discard pellet. 400 µL chilled isopropanol was added and

slowly until white flakes appear and incubated at -20 °C for 1 h. After incubation kept samples in room temperature for 5 min and centrifuged at 10,000 rpm for 10 min. The supernatant was decanted and 400 µL of 95% chilled ethanol and sodium acetate 100 µL was added to the pellet for washing and tapped for 5 min. Supernatant was decanted and dry the pellet in room temperature. Added 20 µL of nuclease free water in tube and stored in -20 °C for further use.

4.5.2 RAPD-PCR amplification

A total of 19 decanucleotide RAPD primers of the series A 3 primers(OPA-05, OPA-12, OPA-20), B 3 primers(OPB-05, OPB-08, OPB-19), E 5 primers(OPE-04, OPE-06, OPE-08, OPE-11, OPE-13) and G 8 primers(OPG-06, OPG-08, OPG-09, OPG-11, OPG-12, OPG-14, OPG-15, OPG-16) obtained from Operon Technologies Inc. (Alameda, CA, USA) primarily screen for identifying primers that was give clear amplification products. RAPD-PCR standardized with respect to the concentration of DNA, RAPD primer, temperature of annealing, TaqDNA Polymerase (Yadav *et al.*, 2017). The PCR reactions carried out in a total volume of 25 µL containing 3 µL of genomic DNA, 3.2 µL (1.25 mM) dNTPs, primer 0.4 µL, PCR buffer 2 µL, 1.5 U of Taq DNA Polymerase (Bangalore Genei, India) of 0.2 µL, and BSA 2 µL, 2.8 µL $MgCl_2$ (25 mM) and 6.4 µL nuclease free water. The PCR cycle conditions for RAPD-PCR included an initial denaturation at 92-95 °C for 5 min followed by 35-39 cycles each of a denaturation step at 94 °C for 4-5 min, 94 °C for 30 S for 1 min; annealing at 32-65 °C for 30 S for 1 min; extension at 72 °C for 1-2 min followed by a final extension at 72 °C for 5-10 min. The PCR products were run in a 1.5 - 2% agarose gel stained with the help of ethidium bromide 3 µL (0.5 µg/mL) (Yadav *et al*., 2017).

4.6 Amplification of the Mitochondrial DNA COI

4.6.1 DNA extraction

DNA was extracted by using Sambrook and Russell (2006) with some modifications. Samples were washed twice with phosphate buffered saline (PBS) (500 µL), centrifuged at 12,000 rpm for 10 min and dried. 300 µL of buffer (100 mM Tris HCl, 50 mM EDTA, 100mM NaCl, 200 mM Sucrose and 1% SDS) and 300 µL of TEX

buffer (1 M Tris HCl, 0.5 M EDTA and 1% Tripton 100) was added respectively, to the sample placed in mortar pestle and crushed the samples. After crushing 10 µL proteinase K (50 mM Tris HCl and 10mM $Cacl_2$) was added in each tube and vortex done vigorously in each tube for 1 min and kept for overnight incubation at 55 °C in thermo cycler at 1300 rpm. The samples were kept in room temperature for 10 min centrifuged at 14,000 rpm for 10 min. Took supernatant in separate tube and added 500 µL of phenol: chloroform: isoamyl alcohol (25:24:1). The solutions were mixed by inverting for 2 min and centrifuged at 14,000 rpm for 10 min. Supernatant was taken in the fresh tubes and discard pellet. 400 µL chilled isopropanol was added and slowly until white flakes appear and incubated at -20 °C for 1 h. After incubation kept samples in room temperature for 5 min and centrifuged at 10,000 rpm for 10 min. The supernatant was decanted and 400 µL of 95% chilled ethanol and sodium acetate 100 µL was added to the pellet for washing and tapped for 5 min. Supernatant was decanted and dry the pellet in room temperature. Added 20 µL of nuclease free water in tube and stored in -20 °C for further use.

The mtDNA COI fragment was amplified from separate weevils using the primer pair COI-LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and COI-HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). The PCR reactions carried out in a total volume of 25 µL containing 2 µL of genomic DNA, 3.2 µL (1.25 mM) dNTPs, LCO1490 primer 0.4 µL, HCO2198 primer 0.4 µL, PCR buffer 2 µL, 1.5 U Taq DNA Polymerase (Bangalore Genei, India) of 0.2 µL, and BSA 2 µL, 2.8 µL $MgCl_2$ (25 mM) and 7 µL nuclease free water. The PCR cycle conditions for RAPD-PCR included an initial denaturation at 94 °C for 5 min followed by 35 cycles each of a denaturation step at 94 °C for 2 min, 94 °C for 30 s; annealing at 51 °C for 30 s; extension at 72 °C for 2 min followed by a final extension at 72 °C for 5 min. The PCR products were run in a 1.5% agarose gel stained with the help of ethidium bromide 3 µL (0.5 µg/mL) (Hebert *et al*., 2004).

4.6.2 COI – PCR amplification

Successful amplified DNA templates were sent for Sanger sequencing on ABI 3730XL sequencer at AgriGenome sequencing facilities (Kochi, Kerala). For Sanger sequencing both directions and fragments were assembled to form contigs by

Geneious V11.0.4 (Kearse *et al*., 2012), then aligned and visually checked for quality and noise to resolve some of the ambiguities. For each sample, we ensured there was no pseudogenes presence similarly to HTS sequences, and we checked for possible cross-contamination by blasting sequences on BOLD to test similarity with conspecific and congeneric existing records. Low quality of electropherograms (potentially due to low DNA concentration, DNA degradation or contaminantion) was discarded. The sequences were deposited in GenBank got the accession numbers for COI (**Table 5**).

Table. 5. Accession number for the CO1 sequences of *Odoiporus longicollis*.

Sl No	**Location**	**Banana cultivars**	***Musa* sp.**	**Accession numbers**
1	Tanhril 1	Changthir	*balbisiana*	KJ446900.1
2	Tanhril 2	Balhlasen	*paradisiaca*	KJ446901.1
3	Tanhril 3	Banria	*paradisiaca*	KJ446902.1
4	Tanhril 4	Vaibalhla	*paradisiaca*	KJ446903.1
5	Tuirial	Changpawl	*paradisiaca*	KJ446904.1
6	Sakawrtuchhum	Changpui	*acuminata*	KJ446905.1
7	Tanhril 5	Vaibalhla	*paradisiaca*	KJ446906.1
8	Tanhril 6	Banria	*paradisiaca*	KJ446907.1
9	Tanhril 7	Banria	*paradisiaca*	KJ446908.1
10	Theriat	Zoblhla	*paradisiaca*	KJ446909.1
11	Theriat	Khumtungbalhla	*paradisiaca*	KJ446910.1
12	Theriat	Khumtungbalhla	*paradisiaca*	KJ446911.1
13	Tuipui	Khumtungbalhla	*paradisiaca*	KJ446912.1
14	Darzo	Khumtungbalhla	*paradisiaca*	KJ446913.1
15	Vanlaiphal	Vaibalhla	*paradisiaca*	KJ446914.1
16	Sangua	Khumtungbalhla	*paradisiaca*	KJ446915.1
17	Cheural	Vaibalhla	*paradisiaca*	KJ446916.1
18	Cheural	Khumtungbalhla	*paradisiaca*	KJ446917.1
19	Rawlbuk	Vaibalhla	*paradisiaca*	KJ446918.1
20	Saiha	Vaibalhla	*paradisiaca*	KJ446919.1

4.7 Sequence analysis by Molecular Evolutionary Genetics Analysis - MEGA

DNA sequence chromatograms were read and discrepancies between forward and reverse sequences were resolved using the Chromas software v 2.01 (http://www.technelysium.com.au/chromas.html). MUSCLE was used to generate the alignments (Edgar, 2004). The sequences were imported into MEGA X for analysis of model test: 0-fold, 2-fold and 4-fold degenerate sites, Estimate of the pattern of nucleotide substitution in COI sequences, estimates of base composition bias, Neutrality analysis: Tajima's test statistics and phylogenetic analysis (Tamura *et al*., 2007). Statistical support for the inferred nodes was obtained by bootstrapping in MEGA X (Kumar *et al*., 2018).

CHAPTER 5

RESULTS

A

B

C

D

E

F

Fig. 1. Wild banana cultivars of Mizoram. **A-** Chnagvandawt (*Musa ornate*), **B-** Changpawl (*Musa paradisiaca*), **C-** Lairawk (*Musa paradisiaca*), **D-** Lairoop (*Musa paradisiaca*), **E-** Changthir (*Musa paradisiaca*) and **F-** Saisu (*Musa paradisiaca*).

Fig. 2. Edible banana cultivars of Mizoram. **A**- Balhlasen (*Musa paradisiaca*), **B**- Banria (*Musa paradisiaca),* **C**- vaibalhla (*Musa paradisiaca*), **D**- Zobalhla (*Musa paradisiaca*), **E**- Kawlbalhla (*Musa paradisiaca*), and **F**- Lawngbalhla (*Musa paradisiaca*).

5.1 Banana cultivars

Nine different banana cultivars were selected for the bioecological studies of ***O. longicollis*** and ***B. subcostatum*** in twenty sites of four districts of Mizoram. The nine banana cultivars were shown (**Table 4)** with their locations, coordinates, common name and scientific name. Both edible and wild cultivars were shown in **Figure 1 and 2.**

5.2 Life cycle of *O. longicollis*

The life stages of *O. longicollis* were fond active throughout the year. Frequent mating was seen throughout day and night. The pre-oviposition period was 20-30 days. The female weevil laid one egg in one air chamber but under the laboratory conditions a bunch of 4-6 eggs at the cut end of the pseudostem inside the air chamber (**Figure 3**). The eggs were yellowish-white and cylindrical with rounded ends. The eggs measures were ranging from 3-4 mm in length and width was 0.9-1.2 mm. The incubation period was varied from 3-6 days in summer and 6-10 days in winter.

The weevil consists 5 instars and the full grown grub is apodous, fleshy and soft body. The head is dark brown colour and not "C" type body. The grubs were measured 14.0-20.0 mm in length with mean 17.25 ± 2.31 mm and 5.0-10.0 mm in width with mean of 7.5 ± 1.8 mm (**Figure 3**). The larval period lasts for 25-35 days in summer with mean of 30 ± 3.5 days and 30-40 days in winter with mean of 35 ± 5.1 days. Under laboratory conditions for banana pseudostem weevil the time period for the development of the egg to the emergence of adult weevil was found 46 ± 7.2 (35 to 58) days in summer (May to August) and 64 ± 11.4 (47 to 85) days in winter (November to February).

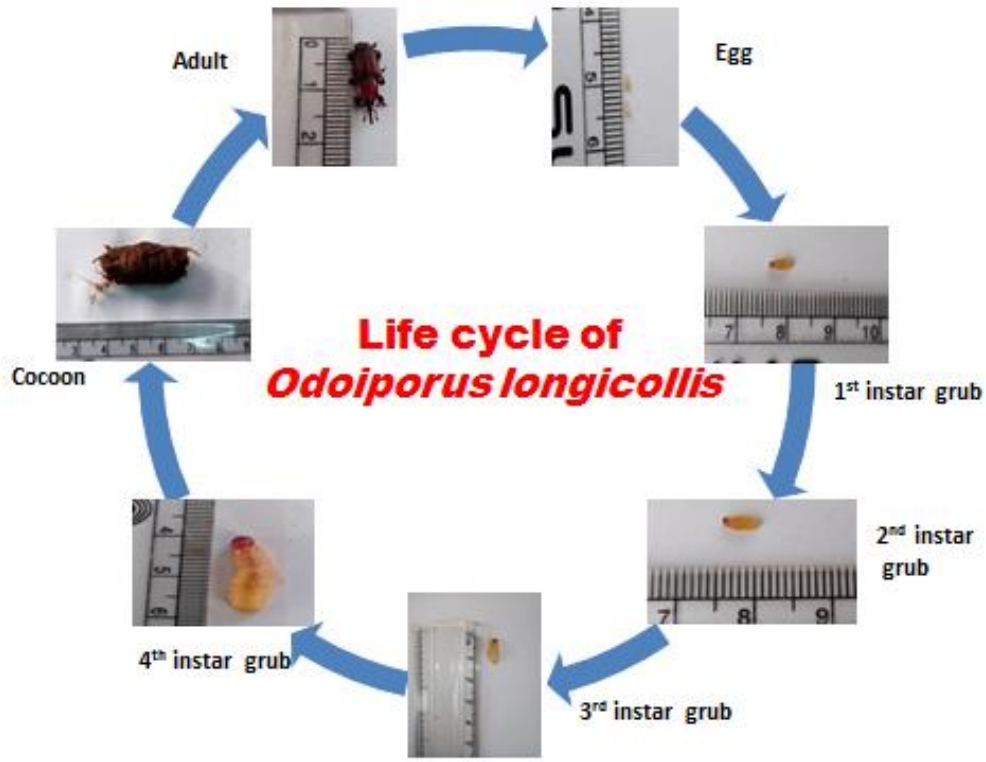

Fig. 3. Life cycle pattern of *O. longicollis* showing egg, 1st , 2nd , 3rd and 4th instar, cocoon, adult life stages.

5.3 Population size and infestation of *O. longicollis* and *B. subcostatum*

5.3.1 Population of *O. longicollis*

Population of *O. longicollis* (August, 2019 to January, 2021) in relation to adult, larva and pupa with three months were displayed in **Figures 5-18**. It shows that the infestation of *O. longicollis* was depending upon the population structure. The population of all stages high in November to July with compare to August to October. The population of ***O. longicollis*** decrease when rainy season starts in Mizoram in the month of May.

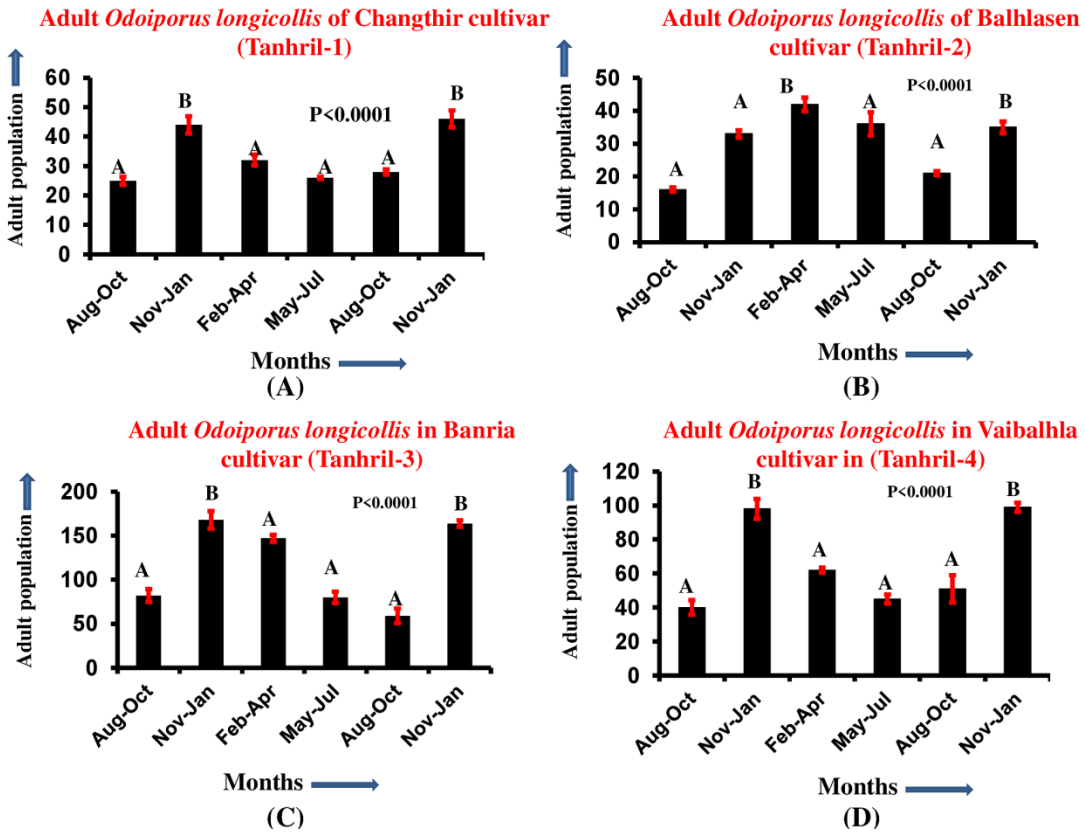

Fig. 4. Adult population dynamics of *Odoiporus longicollis* in the banana cultivars (Changthir, Balhlasen, Banria and Vaibalhla) in Tanhril, Aizawl, Mizoram during August 2019 – January 2021.

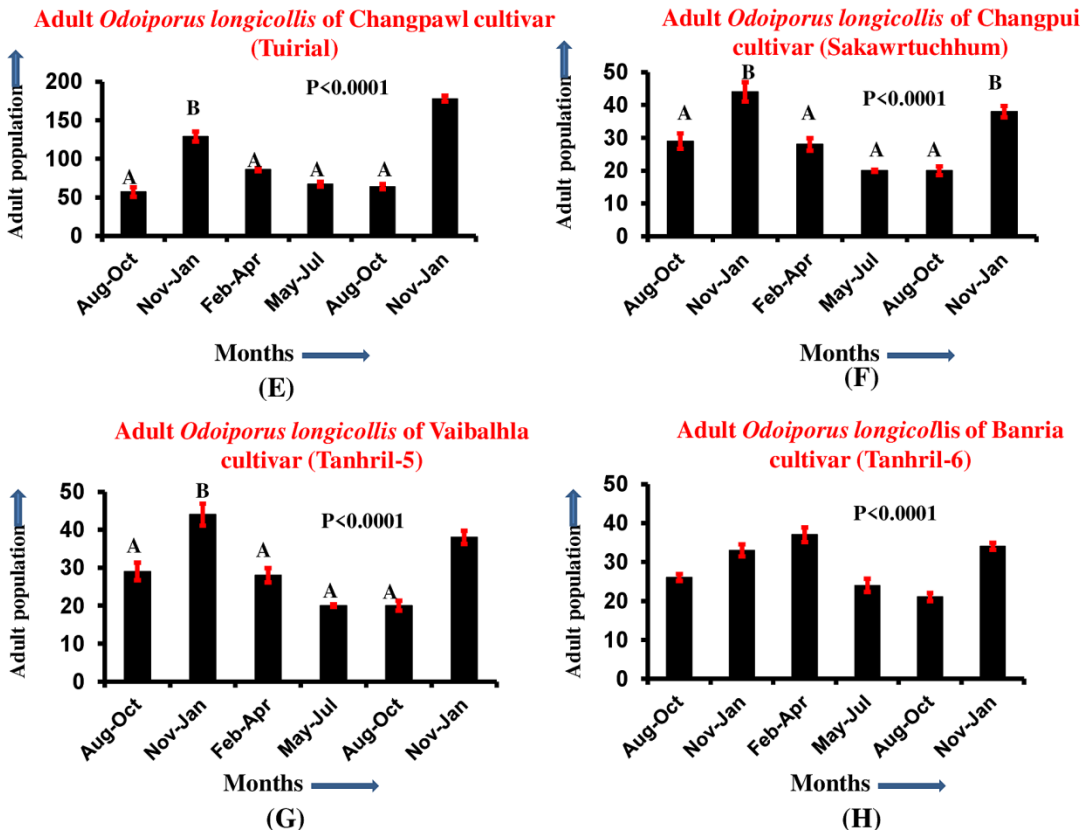

Fig. 5. Adult population dynamics of *Odoiporus longicollis* in the banana cultivars in Tuirial (Changpawl), Sakawrtuchhum (Changpui), Tanhril (Vaibalhla, Banria), Aizawl, Mizoram during August 2019 – January 2021.

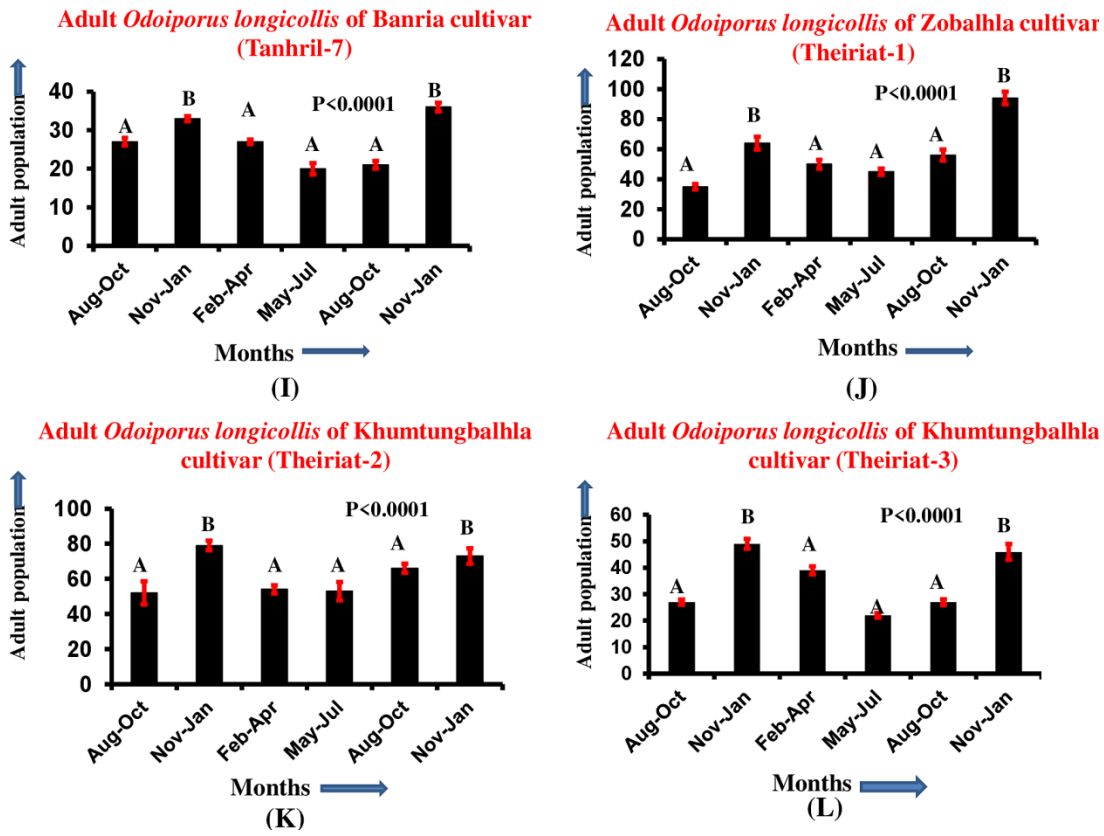

Fig. 6. Adult population dynamics of *Odoiporus longicollis* in the banana cultivars in Tanhril (Banria), Theiriat (Zobalhla, Khumtungbalhla), Aizawl, Mizoram during August 2019 – January 2021.

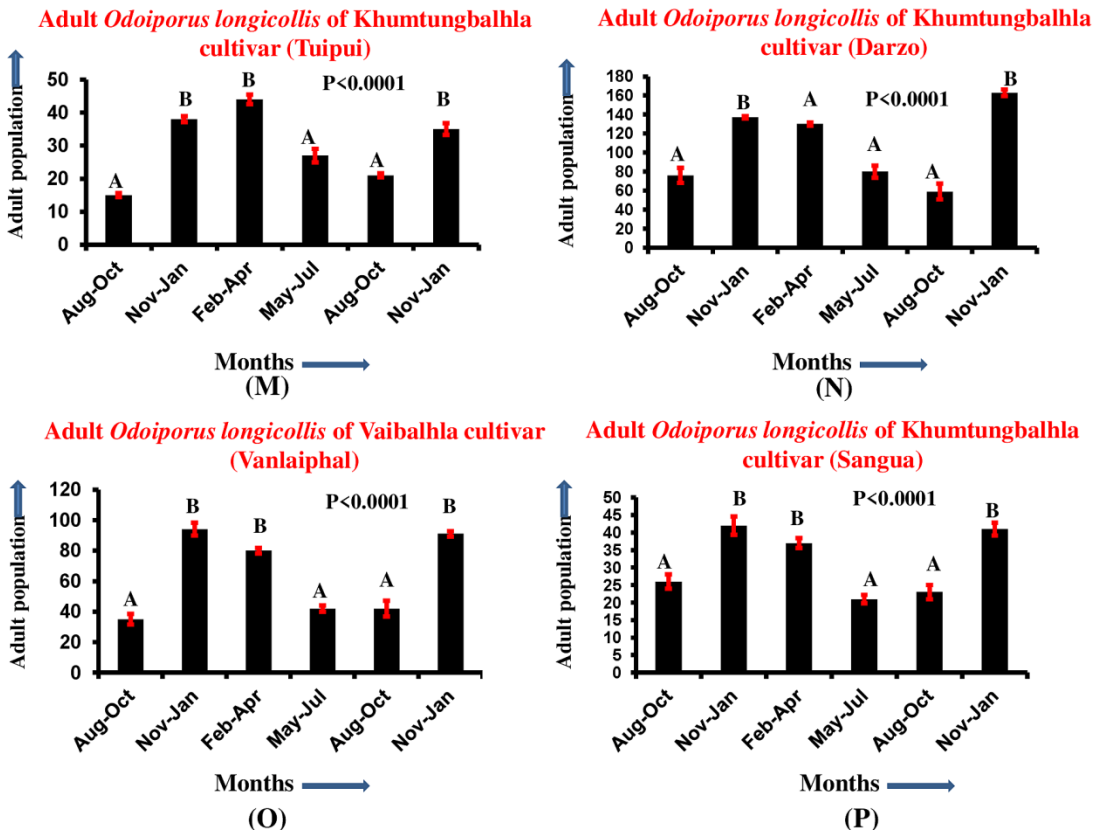

Fig. 7. Adult population dynamics of *Odoiporus longicollis* in the banana cultivars in Tuipui (Khumtungbalhla), Darzo (Khumtungbalhla), Vanlaiphal (Vaibalhla), Sangua (Khumtungbalhla), Aizawl, Mizoram during August 2019 – January 2021.

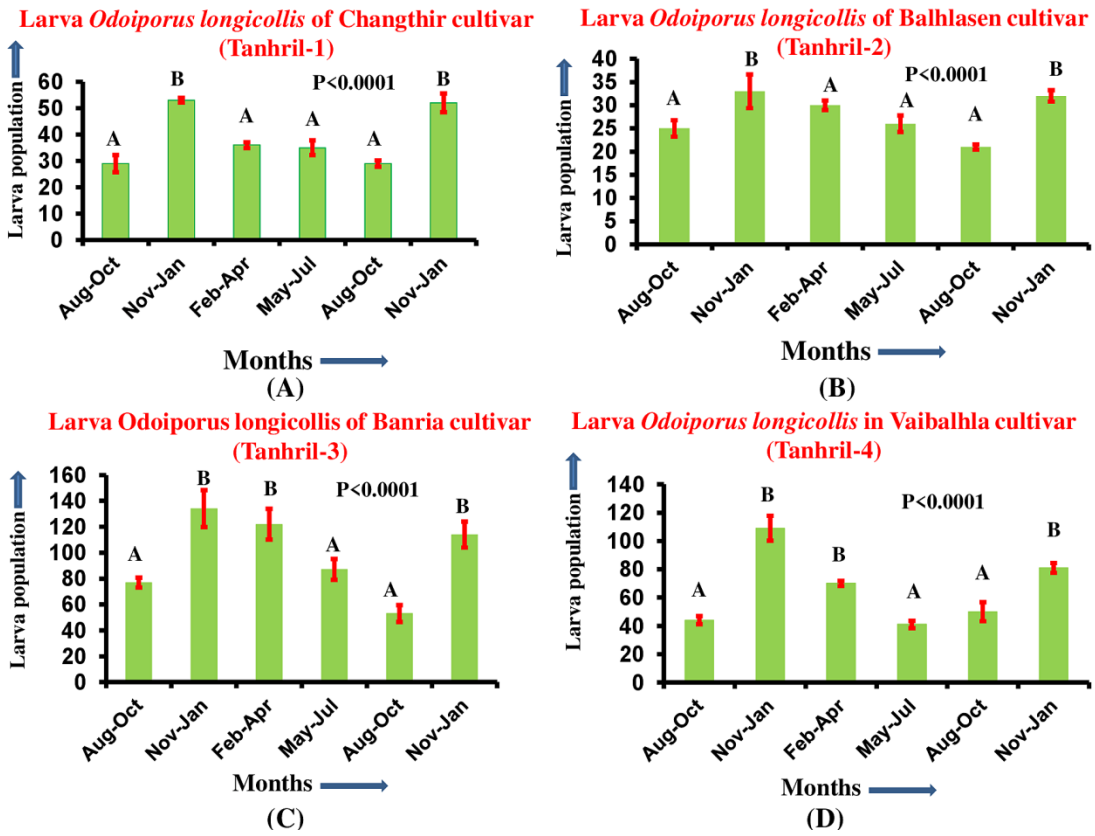

Fig. 8. Larva population dynamics of Odoiporus longicollis in the banana cultivars (Changthir, Balhlasen, Banria and Vaibalhla) in Tanhril, Aizawl, Mizoram during August 2019 – January 2021.

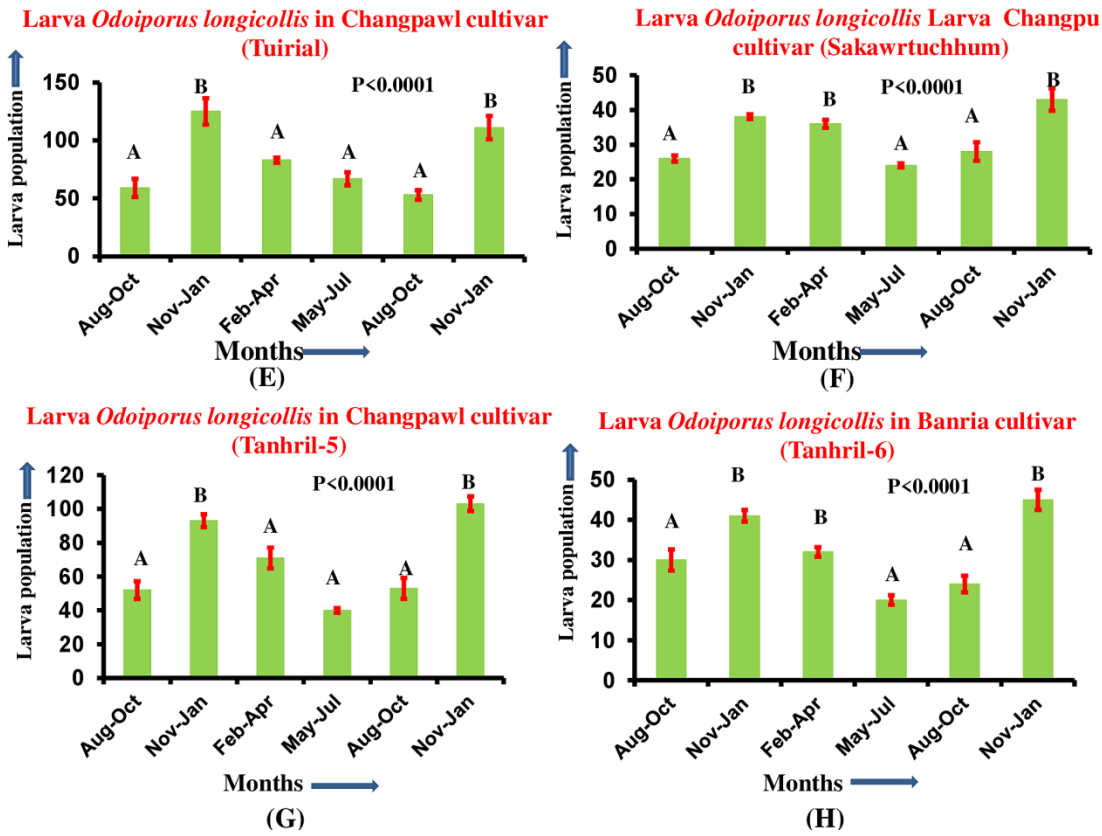

Fig. 9. Larva population dynamics of *Odoiporus longicollis* in the banana cultivars in Tanhril (Changpawl), Sakawrtuchhum (Changpui), Tanhril (Vaibalhla, Banria), Aizawl, Mizoram during August 2019 – January 2021.

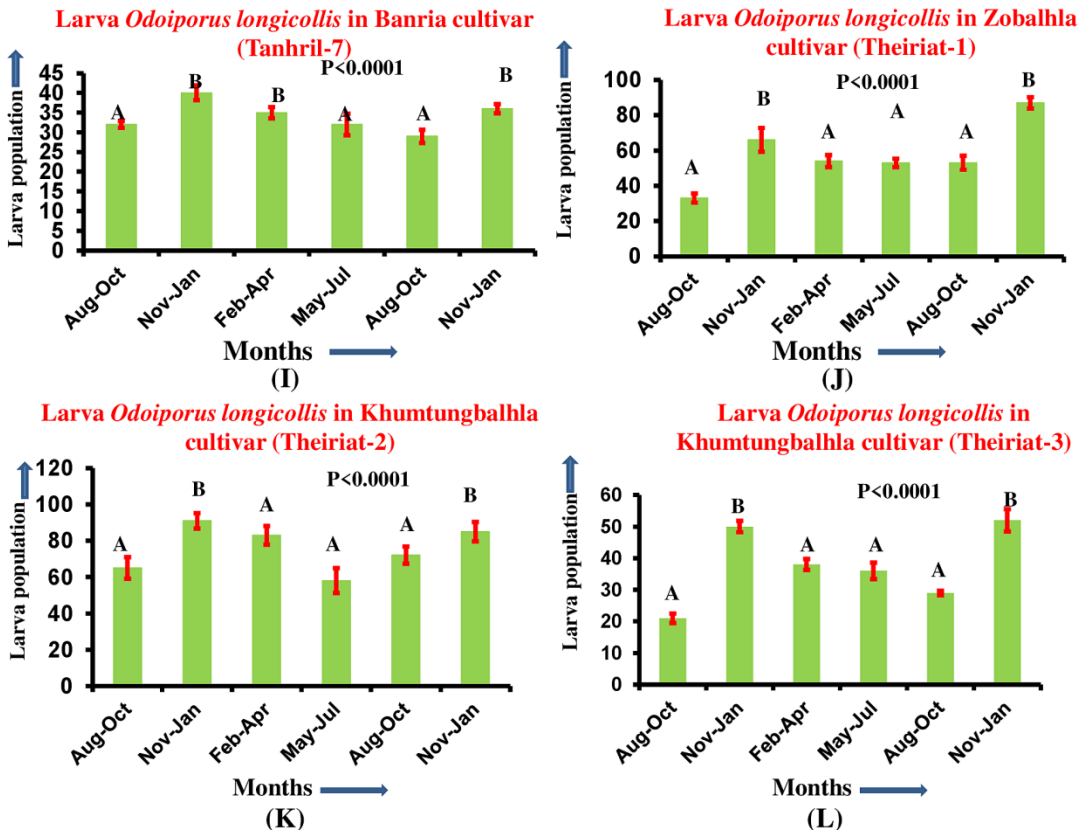

Fig. 10. Larva population dynamics of *Odoiporus longicollis* in the banana cultivars in Tanhril (Banria), Theiriat (Zobalhla, Khumtungbalhla), Tanhril (Vaibalhla, Banria), Aizawl, Mizoram during August 2019 – January 2021.

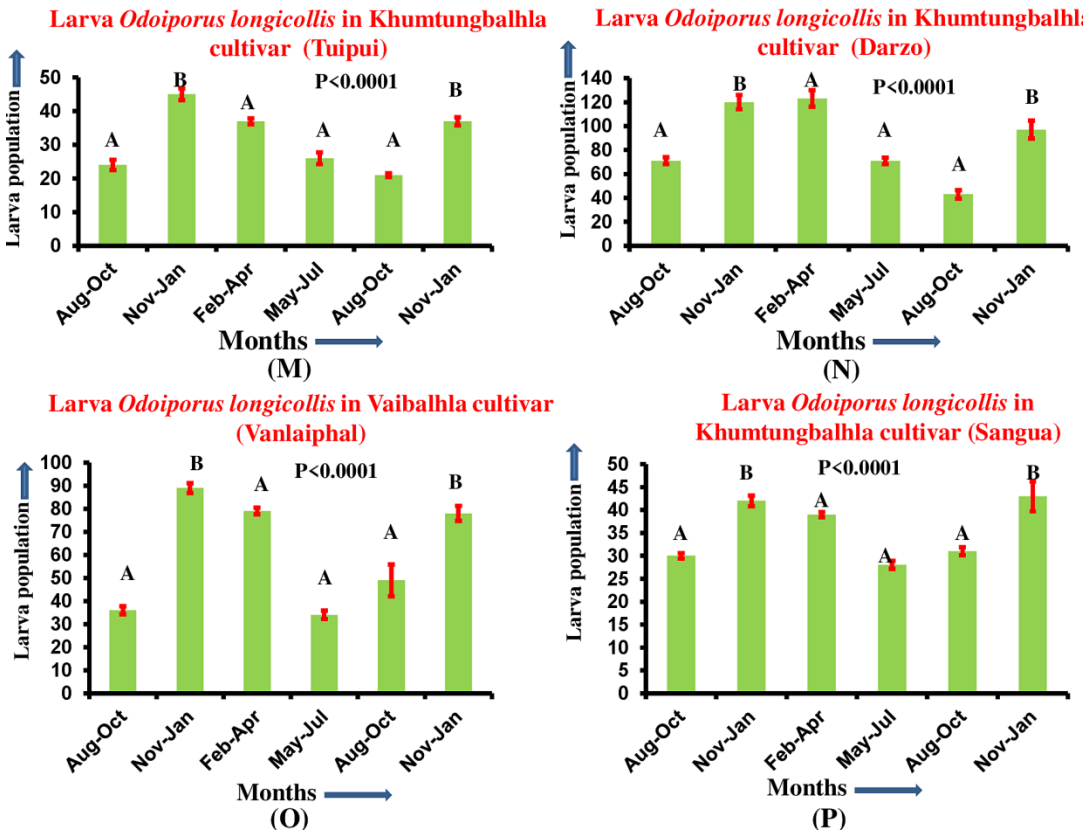

Fig. 11. Larva population dynamics of *Odoiporus longicollis* in the banana cultivars in Tuipui (Khumtungbalhla), Darzo (Khumtungbalhla), Vanlaiphal (Vaibalhla), Aizawl, Mizoram during August 2019 – January 2021.

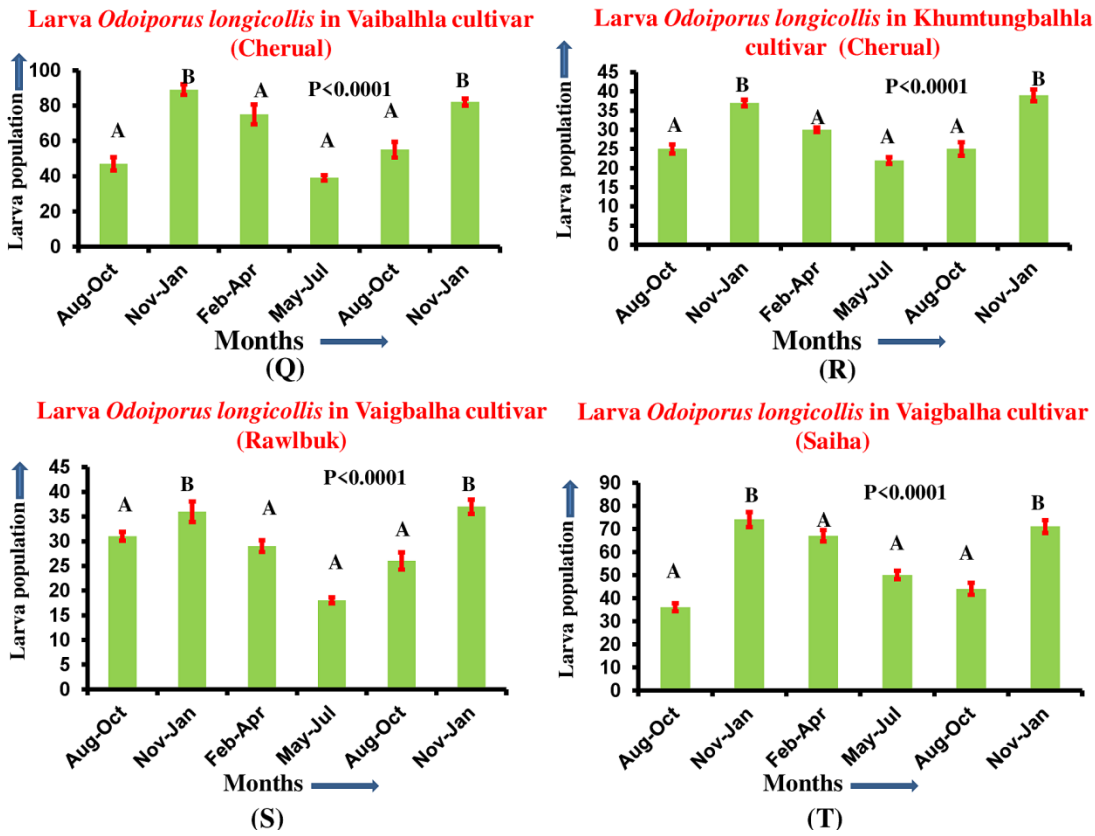

Fig. 12. Larva population dynamics of *Odoiporus longicollis* in the banana cultivars in Cherual (Vaibalhla, Khumtungbalhla), Rawlbuk (Vaibalhla), Saiha (Vaibalhla), Aizawl, Mizoram during August 2019 – January 2021.

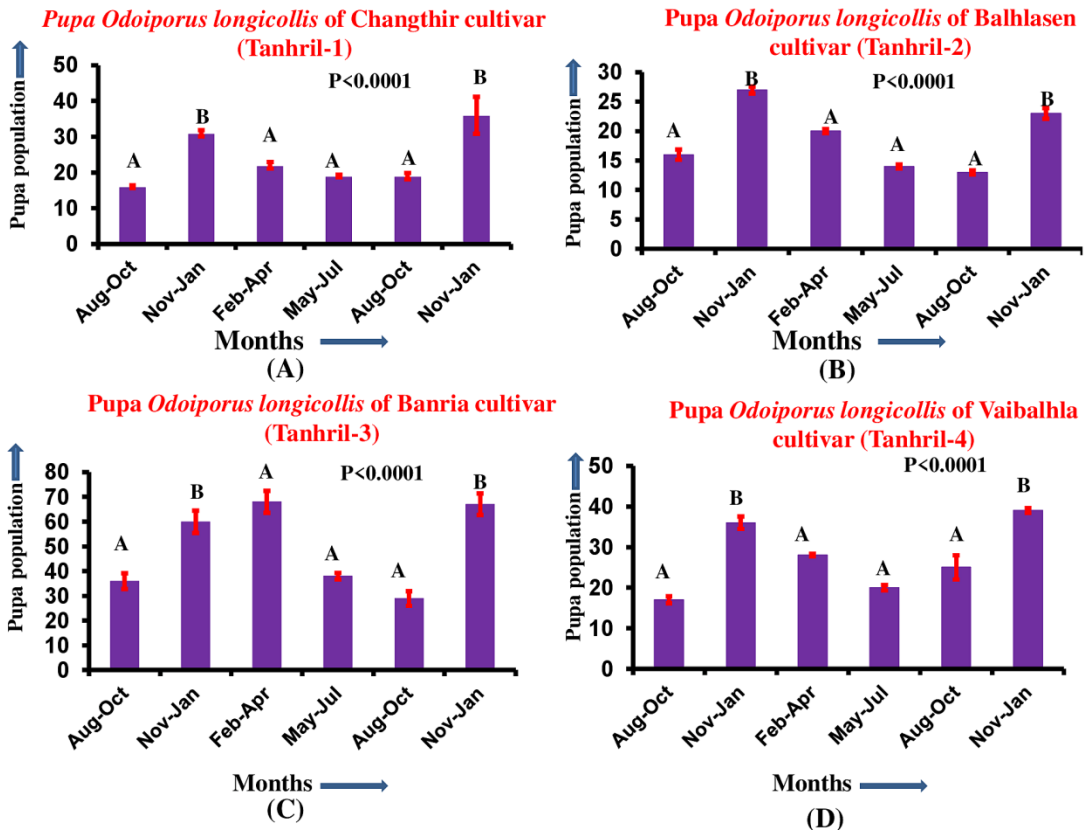

Fig. 13. Pupa population dynamics of Odoiporus longicollis in the banana cultivars (Changthir, Balhlasen, Banria and Vaibalhla) in Tanhril, Aizawl, Mizoram during August 2019 – January 2021.

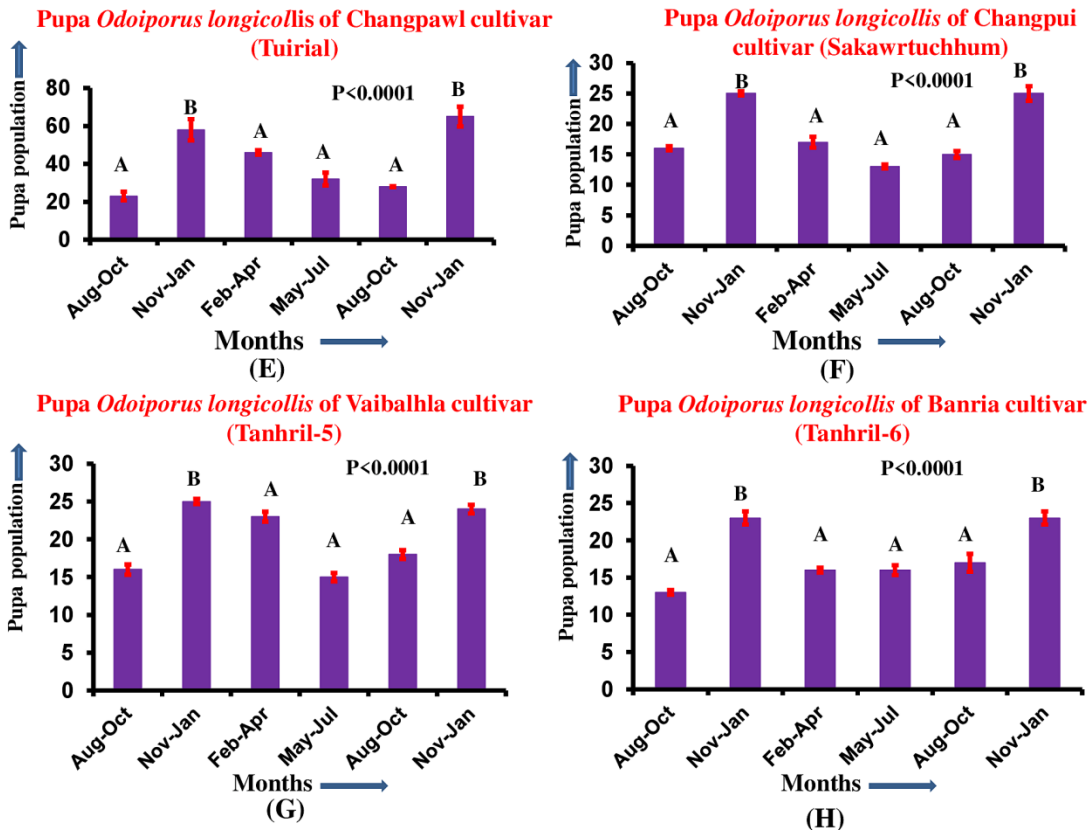

Fig. 14. Pupa population dynamics of *Odoiporus longicollis* in the banana cultivars in Tuirial (Changpawl), Sakawrtuchhum (Changpui), Tanhril (Vaibalhla, Banria), Aizawl, Mizoram during August 2019 – January 2021.

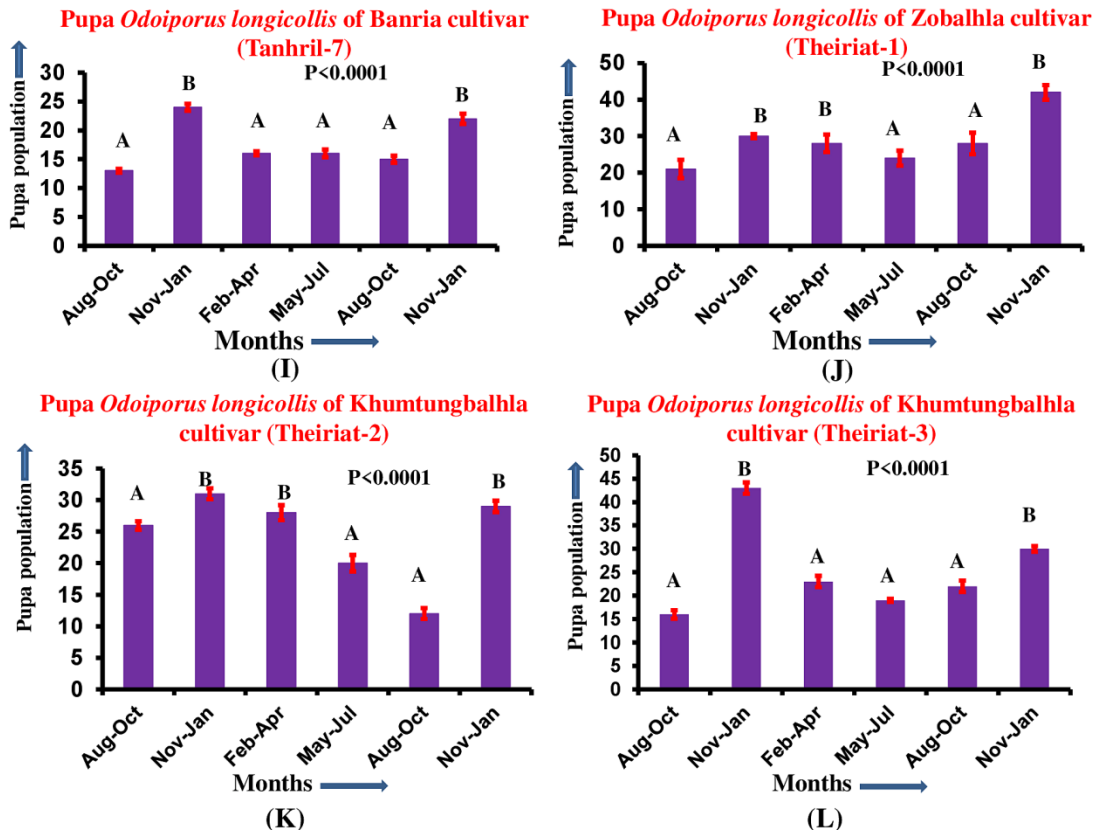

Fig. 15. Pupa population dynamics of *Odoiporus longicollis* in the banana cultivars in Tanhril (Banria), Theiriat (Zobalhla, Khumtungbalhla), Aizawl, Mizoram during August 2019 – January 2021.

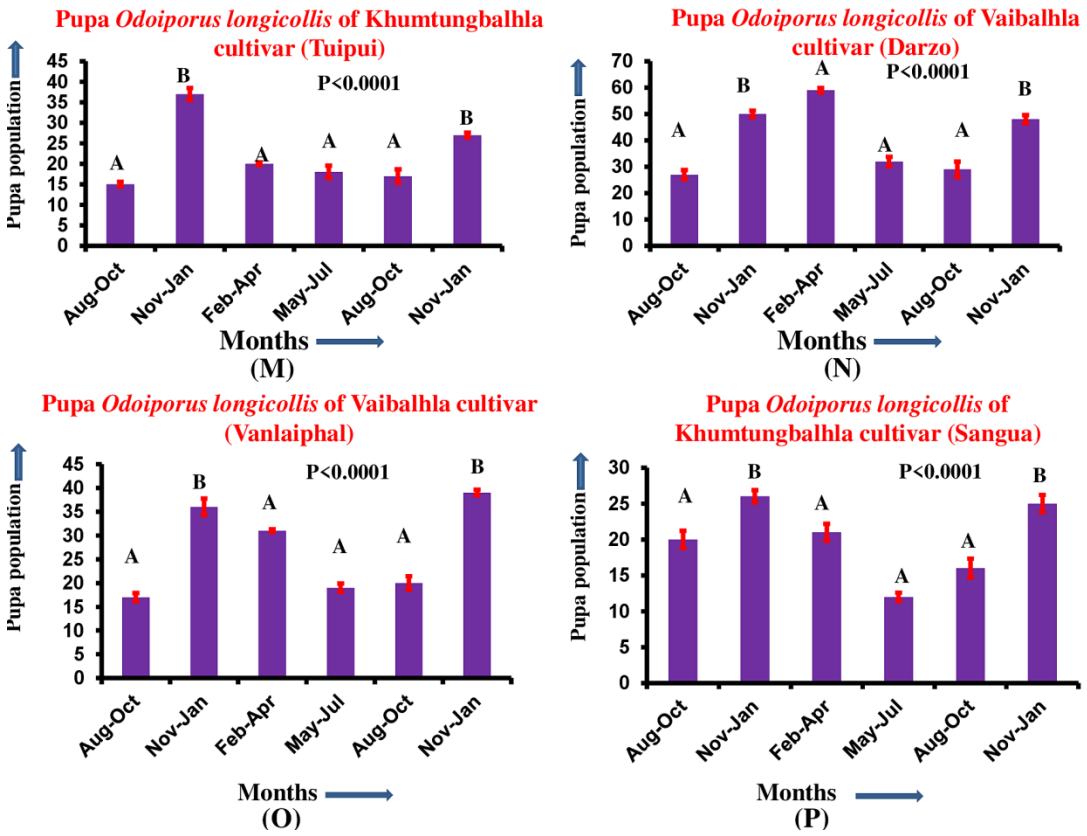

Fig. 16. Pupa population dynamics of *Odoiporus longicollis* in the banana cultivars in Tuipui (Khumtungbalhla), Darzo (Khumtungbalhla), Vanlaiphal (Vaibalhla), Sangua (Khumtungbalhla), Aizawl, Mizoram during August 2019 – January 2021.

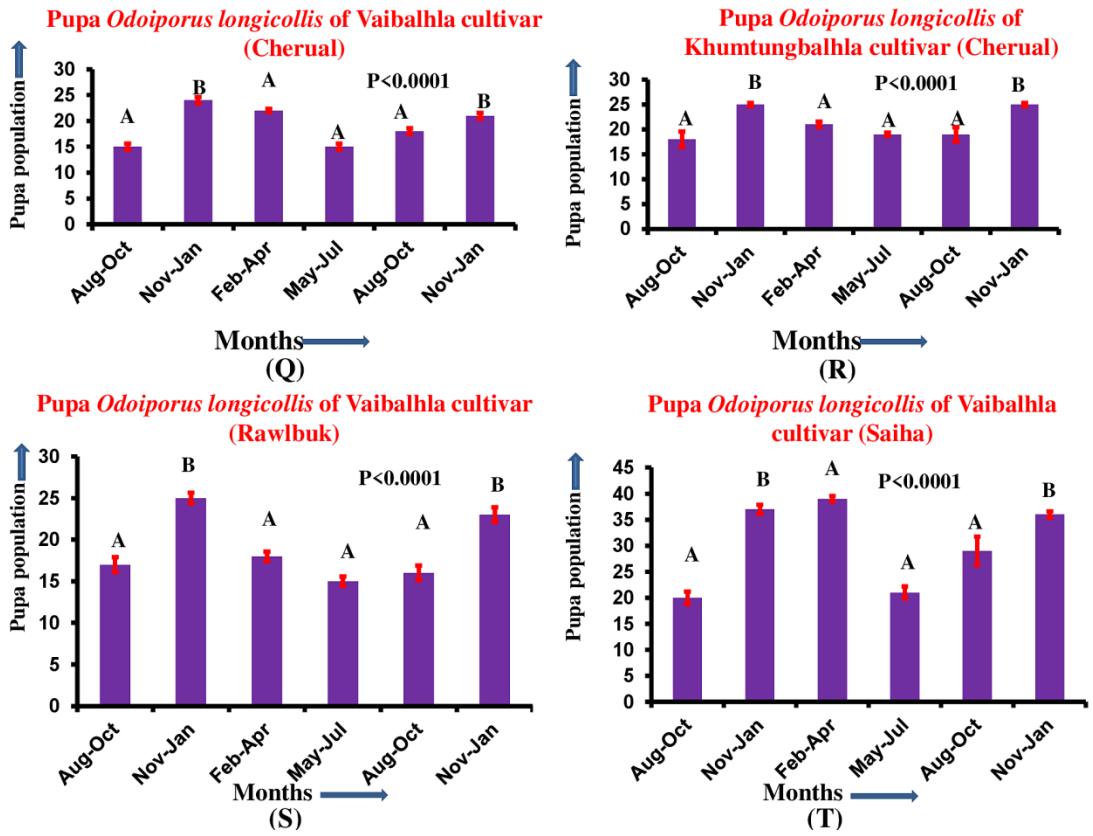

Fig. 17. Pupa population dynamics of *Odoiporus longicollis* in the banana cultivars in Cherual (Vaibalhla, Khumtungbalhla), Rawlbuk (Vaibalhla), Saiha (Vaibalhla), Tanhril (Vaibalhla, Banria), Aizawl, Mizoram during August 2019 – January 2021.

Table. 6. Population and infestation of *O. longicollis* in relation to meteorological parameters.

Months	Mean no. of weevils per plant			Mean no. of holes/ 30 cm^2 of stem area	Maximum temperature (°C)	Minimum temperature (°C)	Rainfall (mm)	Humidity
	Adult	Larva	Pupa					
Aug-19	50.00 ± 1.95	70.00 ± 1.75	10.00 ± 1.5	8.5 ± 0.95	30.00 ± 0.51	17.5 ± 0.25	510.5 ± 5.75	91.00 ± 3.00
Sep-19	70.00 ± 1.5	95.00 ± 2.00	25.00 ± 1.95	8.8 ± 1.00	28.9 ± 0.45	16.8 ± 0.2	321.7 ± 2.75	93.00 ± 0.25
Oct-19	80.00 ± 1.00	85.00 ± 1.95	30.00 ± 2.75	9.2 ± 0.12	29.1 ± 0.45	15.2 ± 0.3	171.4 ± 1.95	90.00 ± 0.95
Nov-19	120.00 ± 1.75	140.00 ± 2.95	41.00 ± 3.25	10.00 ± 0.2	26.00 ± 0.95	14.5 ± 0.5	0.6 ± 0.01	88.00 ± 1.00
Dec-19	150 .00 ± 2.95	165.00 ± 3.95	50.00 ± 1.75	10.7 ± 0.25	26.8 ± 0.25	8.00 ± 0.5	0.12 ± 0.05	86.00 ± 0.25
Jan-20	100.00 ± 1.5	132.00 ± 2.00	55.00 ± 4.25	15.00 ± 0.5	23.00 ± 0.5	6.00 ± 0.2	13.7 ± 0.75	85.00 ± 0.75
Feb-20	125.00 ± 1.75	138.00 ± 3.75	61.0 ± 3.25	16.2 ± 0.8	17.00 ± 0.75	12.00 ± 0.25	2.1 ± 0.1	79.00 ± 0.55
Mar-20	95.00 ± 1.95	120.00 ± 1.75	58.00 ± 2.5	18.5 ± 0.75	29.00 ± 0.95	16.00. ± 0.5	83.4 ± 2.75	77.00 ± 2.00
Apr-20	74.00 ± 1.00	95.00 ± 3.00	41.00 ± 1.25	15.00 ± 0.75	29.5 ± 2.5	15.5 ± 0.95	105.3 ± 3.95	81.00 ± 1.95
May-20	70.00 ± 0.95	97.00 ± 4.15	35.00 ± 2.95	12.5 ± 0.25	30.00 ± 0.75	15.8 ± 0.15	422.3 ± 5.95	83.00 ± 0.55
Jun-20	45.00 ± 3.00	71.00 ± 1.5	27.00 ± 1.95	10.8 ± 0.12	29.00 ± 1.2	16.4 ± 0.19	439.00 ± 5.75	85.00 ± 0.45

Months	Mean no. of weevils per plant			Mean no. of holes/ 30 cm^2 of stem area	Maximum temperature (°C)	Minimum temperature (°C)	Rainfall (mm)	Humidity
	Adult	Larva	Pupa					
Jul-20	51.00 ± 2.95	65.00 ± 2.5	15.00 ± 2.00	5.00 ± 0.1	28.00 ± 0.95	17.00 ± 0.65	372.9 ± 3.75	90.00 ± 2.00
Aug-20	30.00 ± 1.25	54.00 ± 2.95	13.00 ± 1.5	8.00 ± 0.15	30.00 ± 1.00	17.5 ± 0.45	547.9 ± 6.25	89.00 ± 1.75
Sep-20	25.00 ± 1.75	42.00 ± 2.25	15.00 ± 3.00	8.4 ± 0.19	28.9 ± 0.75	18.00 ± 0.55	374.2 ± 2.95	90.00 ± 2.00
Oct-20	45.00 ± 2.5	62.00 ± 1.95	24.00 ± 1.75	9.00 ± 0.25	28.6 ± 0.95	14.00 ± 0.75	165.8 ± 2.75	89.00 ± 0.75
Nov-20	60.00 ± 1.00	104.00 ± 2	40.00 ± 2.5	12.5 ± 0.15	23.00 ± 2.15	11.00 ± 0.25	0.4 ± 0.01	80.00 ± 2.5
Dec-20	85.00 ± 2.75	137.00 ± 2.5	51.00 ± 1.00	13.00 ± 0.25	18.00 ± 1.75	10.00 ± 0.75	0.1 ± 0.01	76.00 ± 0.95
Jan-20	92.00 ± 3.5	148.00 ± 4.25	56.00 ± 1.95	15.9 ± 0.9	17.00 ± 2.00	9.00 ± 0.25	12.1 ± 0.5	74.00 ± 1.5

Population and infestation of *O. longicollis* (August, 2019 to January, 2021) in relation to meteorological parameters were shown in **Table 6**. It shows that the infestation of *O. longicollis* was depends upon the population structure. The infestation of *O. longicollis* was directly proportional with the population of that. It was noticeable from the **Table 6**. In the summer the population of *O. longicollis* was found low in compared to winter and accordingly the infestation of *O. longicollis* also found low in summer and high in winter. It can be concluded that in rainy months the infestation and population of *O. longicollis* decreases.

A negative correlation was observed between the population of adult, pupa and larva of *O. longicollis* and minimum temperature, maximum temperature, rainfall and humidity, respectively. Infestation rate of *O. longicollis* was negatively correlated with minimum temperature, maximum temperature, rainfall and humidity.

The population size and infestation of the *B. subcostatum* were positively related with meteorological parameters. It shows that the infestation of *B. subcostatum* was dependent on the population size. The infestation of *B. subcostatum* increases with the increasing rate of population. The population and infestation rate was highest in the month of August, 2019 and lowest in December, 2020.

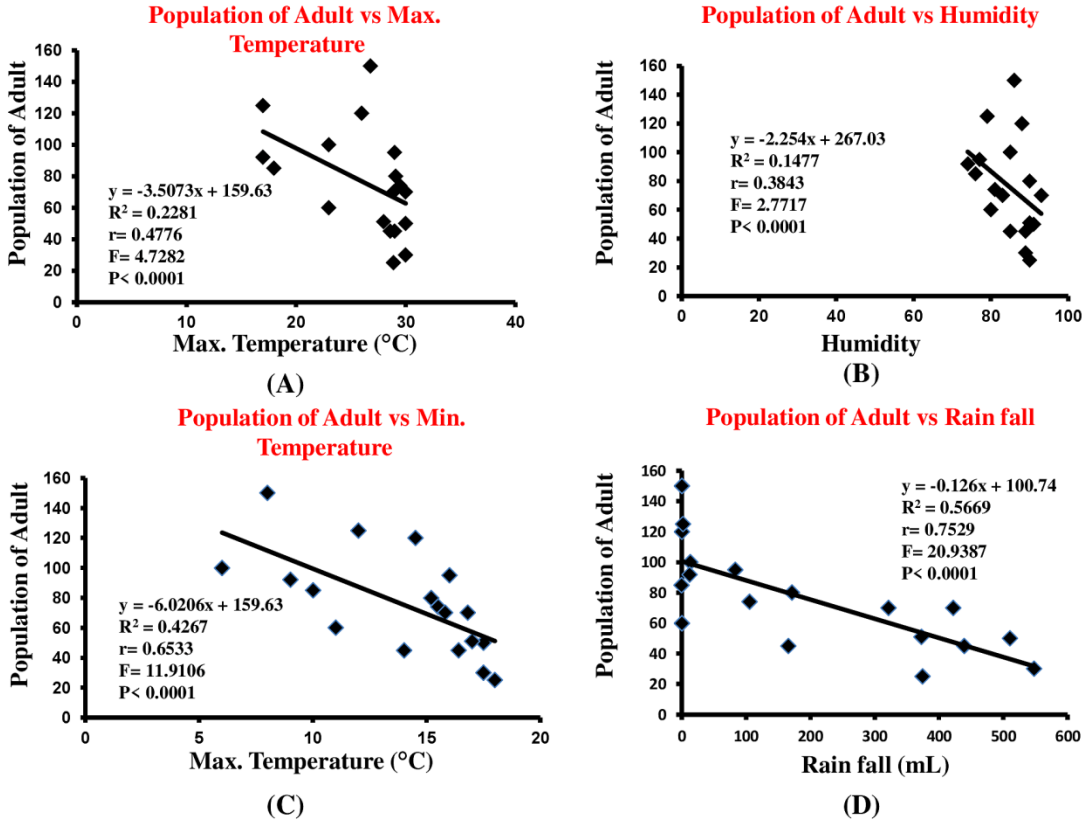

Fig. 18. Correlation and regression analysis between adult population of *O. longicollis* infesting nine banana cultivars in twenty locations of four districts (Aizawl, Lunglei, Lanwgtlai and Saiha) and meteorological parameters (**A**) Population of adult vs Minimum temperature; (**B**) Population of adult vs Maximum temperature; (**C**) Population of adult vs Rainfall and (**D**) Population of adult vs Sunlight.

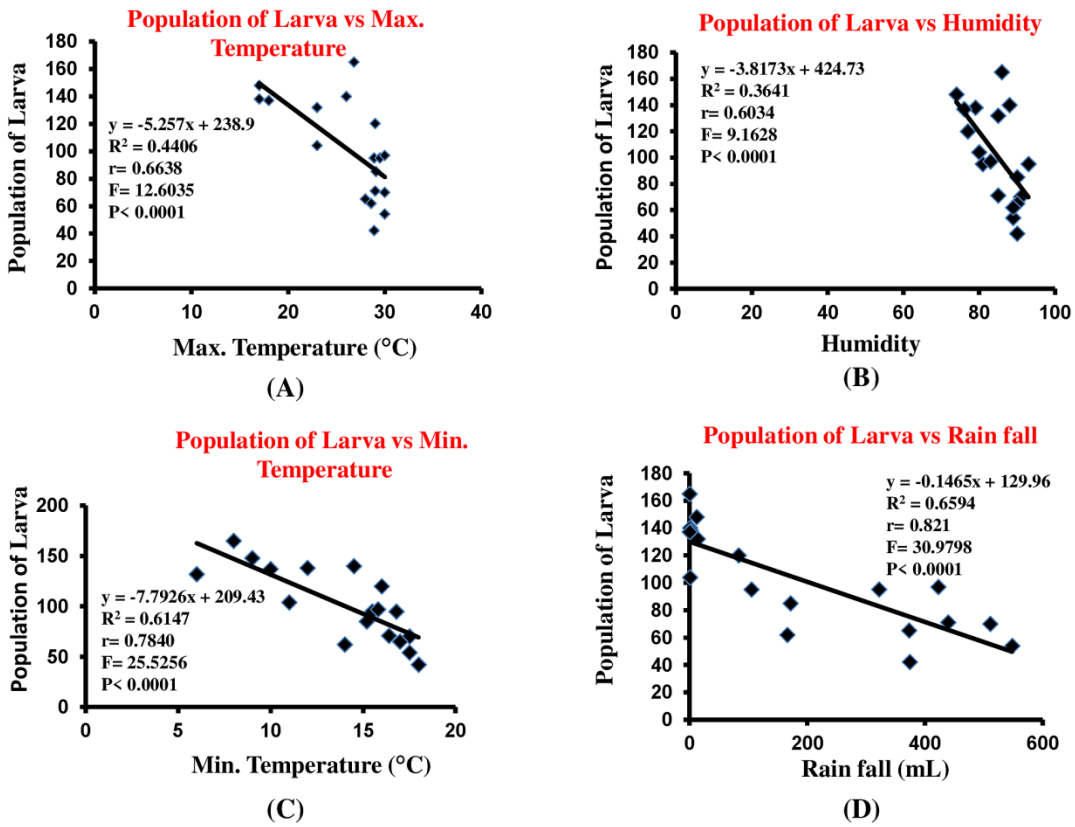

Fig.19. Correlation and regression analysis between larva population of *O. longicollis* infesting nine banana cultivars in twenty locations of four districts (Aizawl, Lunglei, Lanwgtlai and Saiha) and meteorological parameters (**A**) Population of adult vs Minimum temperature; (**B**) Population of adult vs Maximum temperature; (**C**) Population of adult vs Rainfall and (**D**) Population of adult vs Sunlight.

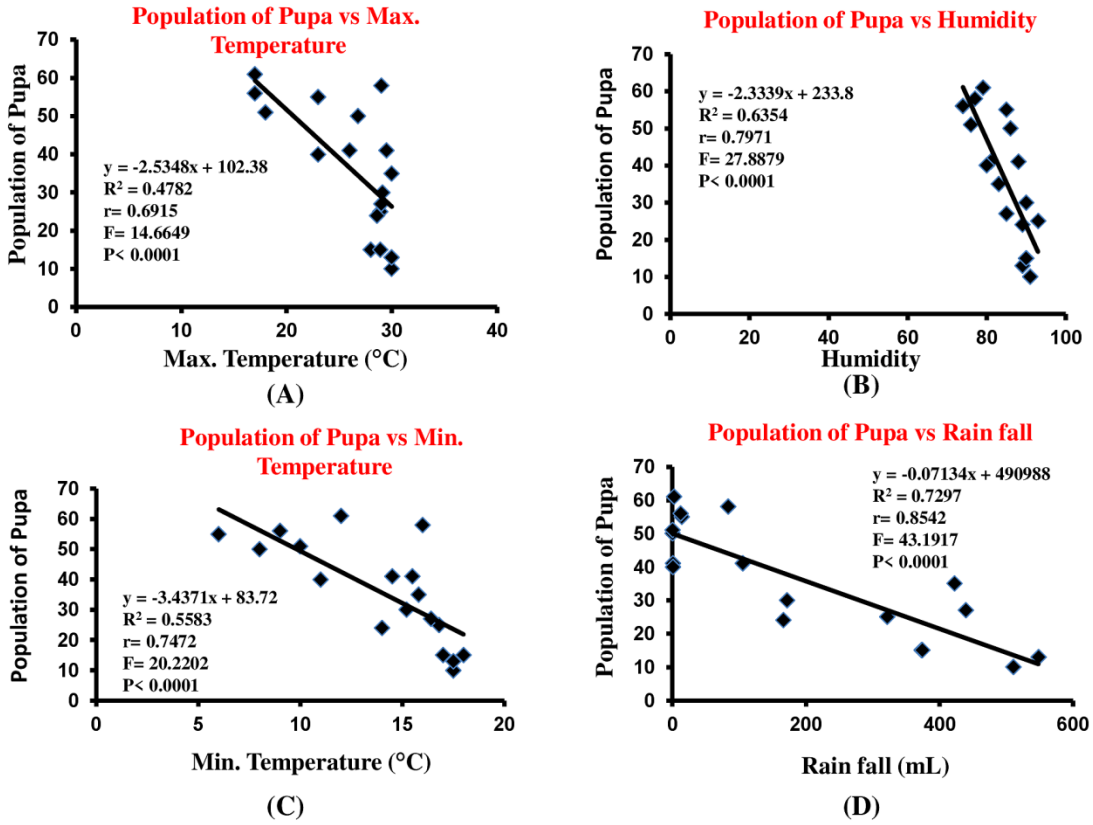

Fig.20. Correlation and regression analysis between pupa population of *O. longicollis* infesting nine banana cultivars in twenty two locations of four districts (Aizawl, Lunglei, Lanwgtlai and Saiha) and meteorological parameters

(**A**) Population of adult vs Minimum temperature,

(**B**) Population of adult vs Maximum temperature,

(**C**) Population of adult vs Rainfall and

(**D**) Population of adult vs Sunlight.

A

B

C

D

E **F**

Fig. 21. Infestation pattern of *O. longicollis* in different banana cultivars.

A, **D** and **F** showing the holes on pseudostem.

B showing the damage in the core of pseudostem done by grubs.

E showing grub making holes in the pseudostem.

Table. 12. Population and infestation of *O. longicollis* in relation to meteorological parameters.

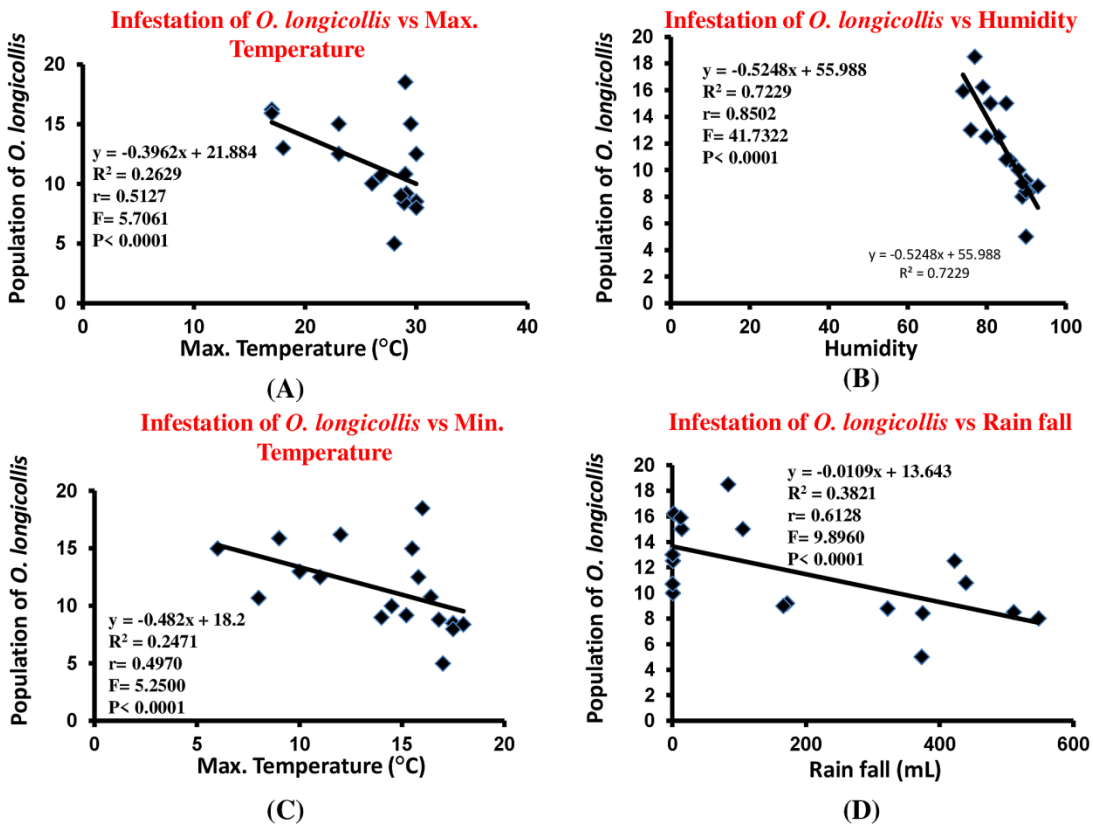

Fig. 22. Correlation and regression analysis between pupa population of *O. longicollis* infesting nine banana cultivars in twenty two locations of four districts (Aizawl, Lunglei, Lanwgtlai and Saiha) and meteorological parameters:

(**A**) Infestation pattern vs Maximum temperature,

(B) Infestation vs humidity,

(C) Infestation vs min. temperature and

(D) Infestation vs Rainfall.

Table. 7. Population dynamics and infestation pattern of *B. subcostatum* collected from nine banana cultivars in twenty two locations of Mizoram and meteorological parameters.

SL No.	Months	Mean no. of beetles per plants	Mean no. of scars/ 5 cm^2 of leaf area	Maximum temperature (°C)	Minimum temperature (°C)	Rainfall (mL)	Humidity
1	August-19	20.00 ± 0.25	18.5 ± 0.25	30.00 ± 0.51	17.5 ± 0.25	510.5 ± 5.75	91.00 ± 3.00
2	September-19	30 ± 0.75	17.4 ± 0.35	28.9 ± 0.45	16.8 ± 0.2	321.7 ± 2.75	93.00 ± 0.25
3	October-19	19.00 ± 0.2	12.7 ± 0.45	29.1 ± 0.45	15.2 ± 0.3	171.4 ± 1.95	90.00 ± 0.95
4	November-19	10.00 ± 0.4	8.7 ± 0.75	26.00 ± 0.95	14.5 ± 0.5	0.6 ± 0.01	88.00 ± 1.00
5	December-19	5.00 ± 0.25	5.1 ± 0.35	26.8 ± 0.25	8.00 ± 0.5	0.12 ± 0.05	86.00 ± 0.25
6	January-20	1.00 ± 0.01	2.3 ± 0.1	23.00 ± 0.5	6.00 ± 0.2	13.7 ± 0.75	85.00 ± 0.75
7	February-20	1.00 ± 0.01	3.1 ± 0.1	17.00 ± 0.75	12.00 ± 0.25	2.1 ± 0.1	79.00 ± 0.55
8	March-20	2.00 ± 0.02	10.1 ± 0.75	29.00 ± 0.95	16.00 ± 0.5	83.4 ± 2.75	77.00 ± 2.00
9	April-20	6.00 ± 0.25	14.5 ± 0.95	29.5 ± 2.5	15.5 ± 0.95	105.3 ± 3.95	81.00 ± 1.95
10	May-20	15.00 ± 1.75	17.6 ± 1.2	30.00 ± 0.75	15.8 ± 0.15	422.3 ± 5.95	83.00 ± 0.55
11	June-20	20.00 ± 25.00	21.4 ± 1.95	29.00 ± 1.2	16.4 ± 0.19	439.00 ± 5.75	85.00 ± 0.45
12	July-20	30.00 ± 3.5	29.5 ± 2.2	28.00 ± 0.95	17.00 ± 0.65	372.9 ± 3.75	90.00 ± 2.00

SL No.	Months	Mean no. of beetles per plants	Mean no. of scars/ 5 cm^2 of leaf area	Maximum temperature (°C)	Minimum temperature (°C)	Rainfall (mL)	Humidity
13	August-20	31.00 ± 3.00	30.7 ± 2.95	30.00 ± 1.00	17.5 ± 0.45	547.9 ± 6.25	89.00 ± 1.75
SL No.	Months	Mean no. of beetles per plants	Mean no. of scars/5 cm^2 of leaf area	Maximum temperature (°C)	Minimum temperature (°C)	Rainfall (mL)	Humidity
14	September-20	29.00 ± 2.5	27.5 ± 1.5	28.9 ± 0.75	18.00 ± 0.55	374.2 ± 2.95	90.00 ± 2.00
15	October-20	19.00 ± 2.1	15.4 ± 0.95	28.6 ± 0.95	14.00 ± 0.75	165.8 ± 2.75	89.00 ± 0.75
16	November-20	10.00 ± 0.2	8.1 ± 0.5	23.00 ± 2.15	11.00 ± 0.25	0.4 ± 0.01	80.00 ± 2.5
17	December-20	5.00 ± 0.1	3.1 ± 0.2	18.00 ± 1.75	10.00 ± 0.75	0.1 ± 0.01	76.00 ± 0.95
18	January-21	4.00 ± 0.1	2.4 ± 0.1	17.00 ± 2.00	9.00 ± 0.25	12.1 ± 0.5	74.00 ± 1.5

The population of *B. subcostatum* positively correlated with minimum temperature, maximum temperature, rainfall and humidity. With the increasing rate of population of *B. subcostatum* the infestation rate also increases. Hence, the rate of infestation of *B. subcostatum* was also positively correlated with minimum temperature, maximum temperature, rainfall and humidity (**Table 7**).

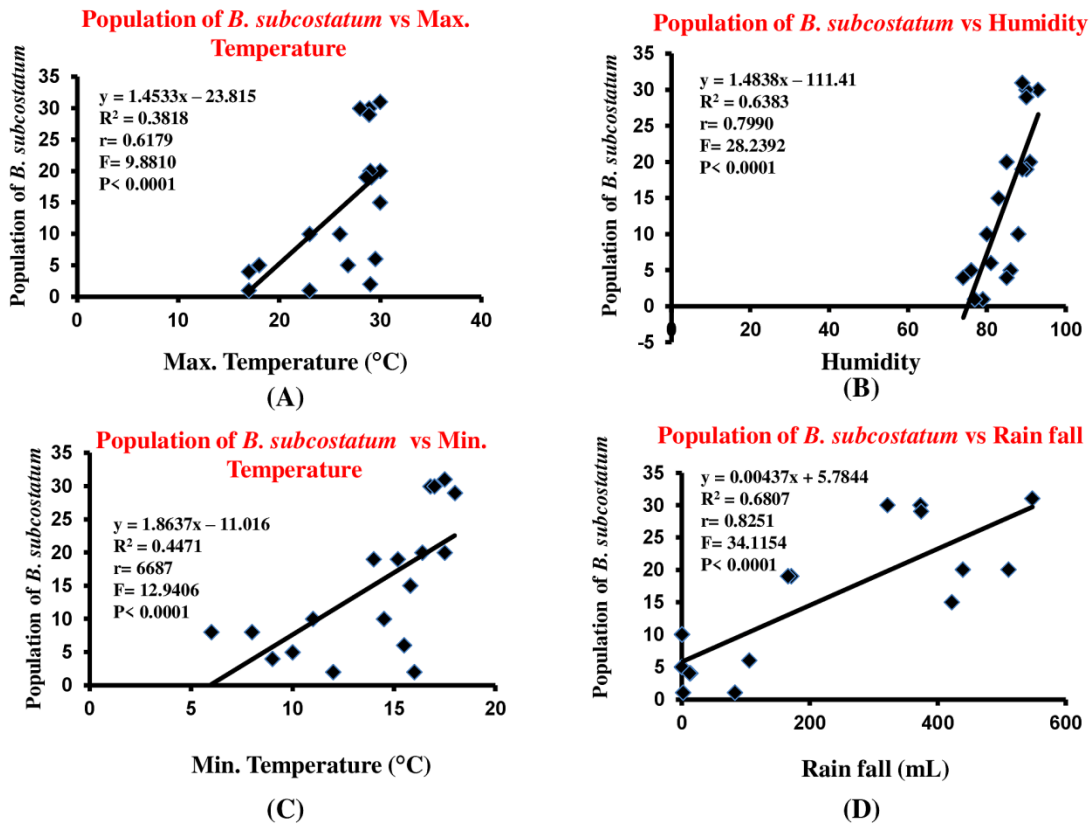

Fig. 23. Correlation and regression analysis between total population of *B. subcostatum* infesting nine banana cultivars in six locations of Aizawl district and meteorological parameters

(**A**) Population of *B. subcostatum* vs Minimum temperature,

(**B**) Population of *B. subcostatum* vs Maximum temperature,

(**C**) Population of *B. subcostatum* vs Rainfall and

(**D**) Population of *B. subcostatum* vs Sunlight.

A

Fig. 24. *B. subcostatum* on banana leaf

B

Fig. 25. Figure showing infestation of *B. subcostatum* banana.

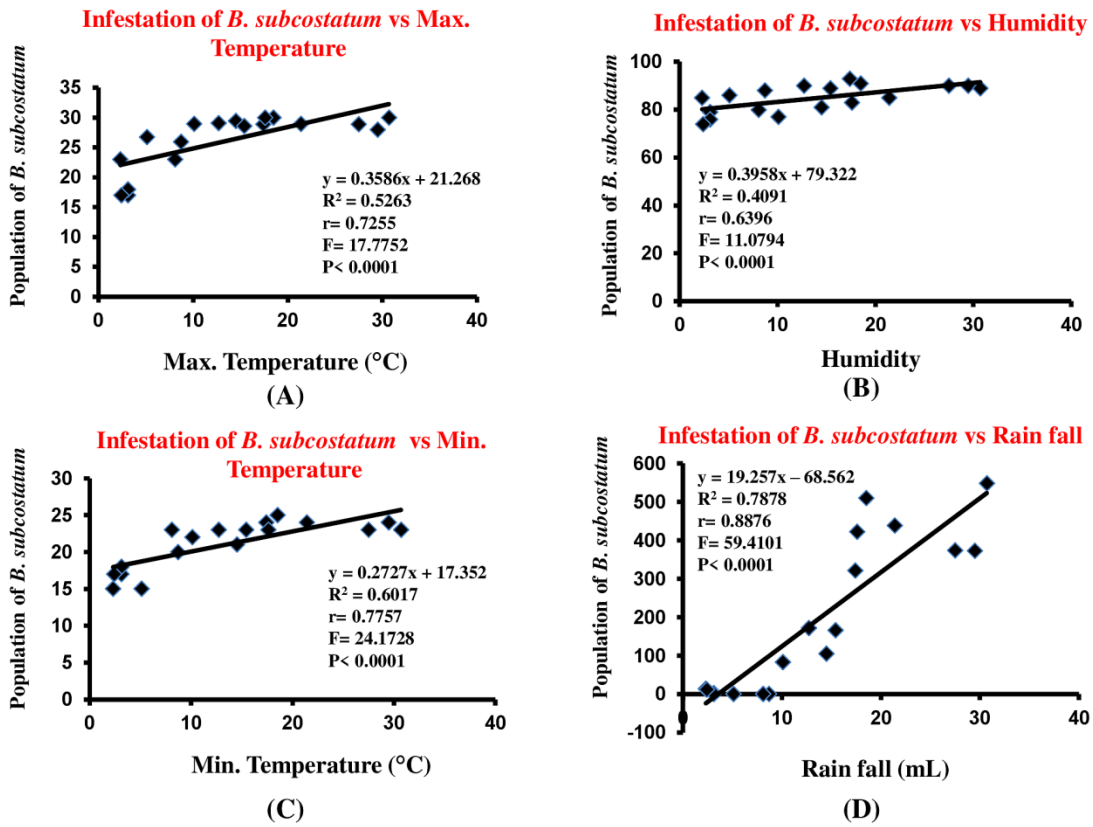

Fig. 26. Correlation and regression analysis between infestation of *B. subcostatum* infesting nine banana cultivars in six locations of Aizawl district and meteorological parameters:

(**A**) Infestation of *B. subcostatum* vs Minimum temperature,

(**B**) Infestation of *B. subcostatum* vs Maximum temperature,

(**C**) Infestation of *B. subcostatum* vs Rainfall and

(**D**) Infestation of *B. subcostatum* vs Sunlight.

5.4 Random Amplified Polymorphic DNA fingerprints of *Odoiporus longicollis* and *Basilepta subcostatum*.

- The randomly amplified polymorphic DNA (RAPD) marker study displayed no amplification results with 19 primers both in *O. longicollis* and *B. subcostatum*.
- Because of no results obtained in RAPD analysis as an alternative COI marker was chosen for population genetic structure analysis of *O. longicollis* and *B. subcostatum*.

5.5 Model test for Substitution pattern of COI sequences of *O. longicollis* and *B. subcostatum*

Analysis of 20 nucleotide sequences comprising 835 positions using model test revealed that out of Maximum Likelihood fits of 24 different nucleotide substitution models the best fit model was Tamura-3-Parameter.The best fit model was assigned based on the following criteria: BIC-2836.83 , AIC- 2535.821292 and lnL -1228.81 score. Transition/Transversion bias (R) is 8.02 (**Table 8**). The substitution site rate variation under Gamma parameter category-1 was observed to be high in A and T (0.34) while low in C and G (0.15) (**Table 8**).

Analysis of 6 nucleotide sequences comprising 674 positions using model test revealed that out of Maximum Likelihood fits of 24 different nucleotide substitution models the best fit model was Tamura-3-Parameter.The best fit model was assigned based on the following criteria: BIC-2814.01, AIC- 2746.872350 and lnL -1362.39 score. Transition/Transversion bias (R) is 1.27. The substitution site rate variation under Gamma parameter category-1 was observed to be high in A and T (0.32) while low in C and G (0.17) (**Table 8**).

Table. 8. Model test for Substitution pattern of CO1 of *O. longicollis* pattern in COI sequences of *O. longicollis*.

Model test	Parameters	Value for *O. longicollis*	Value for *B. subcostata*
Tamura -3-parameter	Bayesian Information Criterion (BIC)	2836.837413	2814.010787
	Akaike Information Criterion, corrected (AICc)	2535.821292	2746.872350
	Maximum Likelihood value (lnL)	-1228.817009	-1362.396392
	Evolutionarily invariable sites(+I)	n/a	n/a
	Discrete Gamma distribution (+G)	n/a	n/a
	Transition/Transversion bias (R)	8.024666425	1.2797985117
Gamma parameter catagory 1	f(A)	0.347874251	0.3270270270
	f(T)	0.347874251	0.3270270270
	f(C)	0.152125749	0.1729729729
	f(G)	0.152125749	0.1729729729
	r(AT)	0.02	0.07
	r(AC)	0.01	0.04
	r(AG)	0.14	0.1
	r(TA)	0.02	0.07
	r(TC)	0.14	0.1
	r(TG)	0.01	0.04
	r(CA)	0.02	0.07
	r(CT)	0.31	0.19
	r(CG)	0.01	0.04
	r(GA)	0.31	0.19
	r(GT)	0.02	0.07
	r(GC)	0.01	0.04

5.5.1 Conserved, Variable, Parsimony and Singleton sites

Out of 835 nucleotides of the CO1 gene in O. longicollis we have found 817 conserved sites, 18 variable sites, and 8 parsimony informative sites, 10 singleton sites and in case of scarring beetle in 674 nucleotides 396 conserved sites, variables sites 159, 1 parsimony informative sites, 158 singleton sites. The COI region showed

no insertion or deletion. The base composition of this COI fragment varied amongst the individuals.

5.5.2 0, 2 and 4-fold degenerate sites

Analysis of degeneracy of 20 geographical populations of O. longicollis COI sequences showed 532 0-fold degenerate sites which are non-synonymous followed by 180 2-fold degenerate sites and 117 4-fold degenerate sites which are synonymous substitutions, where as in scarring beetle out of 6 geographical populations 412 0-fold degenerate sites which are non-synonymous followed by 99 2-fold degenerate sites and 97 4-fold degenerate sites.

5.5.3 Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution in COI sequences of *O. longicollis* and scarring beetle of geographical populations and banana cultivars

Table. 9. Nucleotide Substitution in COI sequences of *O. longicollis* and *B. subcostatum* of geographical populations and banana cultivars.

O. longicollis					***B. subcostatum***				
	A	**T**	**C**	**G**		**A**	**T**	**C**	**G**
A	**-**	*3.43*	*1.44*	**5.41**	**A**	**-**	*4.09*	*2.02*	**0.03**
T	*2.68*	**-**	**19.28**	*1.23*	**T**	*4.38*	**-**	**24.52**	*2.38*
C	*2.68*	**45.99**	**-**	*1.23*	**C**	*4.38*	**49.64**	**-**	*2.38*
G	**11.75**	*3.43*	*1.44*	**-**	**G**	**0.06**	*4.09*	*2.02*	**-**

Each entry shows the probability of substitution (r) from one base (row) to another base (column) (Nei and Kumar, 2000). For simplicity, the sum of r values is made equal to 100. Rates of different transitional substitutions are shown in bold and those of transversionsal substitutions are shown in italics (**Table 9**). This analysis involved 20 nucleotide sequences for *O. longicollis* and 6 nucleotides in case of *B. subcostatum*. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 835 positions in the final dataset for *O. longicollis* and 674 positions for *B. subcostatum* (**Table 9**). Evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018).

5.5.4 Estimates of base composition bias difference between geographical populations and banana cultivars

The difference in base composition bias per site is shown. Note that even when the substitution patterns are homogeneous among lineages, the compositional distance will correlate with the number of differences between sequences. This analysis involved 20 nucleotide sequences in *O. longicollis* and 6 nucleotide sequences in scarring beetle. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 835 and 674 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

5.5.5 Disparity Index Test of the Homogeneity of Substitution Patterns between Sequences of mitochondrial COI in *O. longicollis* and *B. subcostatum*

The difference in base composition bias per site is shown. Note that even when the substitution patterns are homogeneous among lineages, the compositional distance will correlate with the number of differences between sequences. This analysis involved 20 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding.There were a total of 835 positions **(Table 11)** in the final dataset. Evolutionary analyses were conducted in MEGA X.

Table. 10. Disparity Index of *O. longicollis*.

	ASC	AT1V	AT2V	AT3B	AT4V	AT5B	AT6CT	ATC	KTRV	LTR2BK	LTR3Z	LTPKB	LDKTB	LSVB	LNSG1B	LNCH1V	LNCH2K	LRLV	LNSG2Z	SSHV
ASC																				
AT1V	0.001																			
AT2V	0.001	0.000																		
AT3B	0.001	0.000	0.000																	
AT4V	0.001	0.004	0.004	0.004																
AT5B	0.001	0.001	0.001	0.001	0.001															
AT6CT	0.001	0.004	0.004	0.004	0.000	0.001														
ATC	0.001	0.001	0.001	0.001	0.001	0.000	0.001													
KTRV	0.001	0.000	0.000	0.000	0.004	0.001	0.004	0.001												
LTR2BK	0.001	0.000	0.000	0.000	0.004	0.001	0.004	0.001	0.000											
LTR3Z	0.001	0.000	0.000	0.000	0.004	0.001	0.004	0.001	0.000	0.000										
LTPKB	0.001	0.000	0.000	0.000	0.004	0.001	0.004	0.001	0.000	0.000	0.000									
LDKTB	0.001	0.000	0.000	0.000	0.004	0.001	0.004	0.001	0.000	0.000	0.000	0.000								
LSVB	0.001	0.004	0.004	0.004	0.002	0.004	0.002	0.004	0.004	0.004	0.004	0.004	0.004							
LNSG1B	0.006	0.012	0.012	0.012	0.004	0.008	0.004	0.008	0.012	0.012	0.012	0.012	0.012	0.004						
LNCH1V	0.002	0.006	0.006	0.006	0.001	0.004	0.001	0.004	0.006	0.006	0.006	0.006	0.006	0.001	0.004					
LNCH2K	0.002	0.001	0.001	0.001	0.004	0.001	0.004	0.001	0.001	0.001	0.001	0.001	0.001	0.004	0.011	0.005				
LRLV	0.001	0.004	0.004	0.004	0.002	0.004	0.002	0.004	0.004	0.004	0.004	0.004	0.004	0.000	0.004	0.001	0.004			
LNSG2Z	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.006	0.002	0.010	0.004		
SSHV	0.002	0.006	0.006	0.006	0.004	0.006	0.004	0.006	0.006	0.006	0.006	0.006	0.006	0.004	0.006	0.005	0.010	0.004	0.002	

Table. 11. Disparity Index of *B. subcostatum*.

	AZ_SITE1	AZ_SITE2	AZ_SITE3	AZ_SITE4	AZ_SITE5	AZ_SITE6
AZ_SITE1						
AZ_SITE2	0.00000					
AZ_SITE3	0.00000	0.00000				
AZ_SITE4	0.00000	0.00000	0.00000			
AZ_SITE5	0.00000	0.00180	0.00180	0.00180		
AZ_SITE6	0.58257	0.56422	0.56422	0.56422	0.63761	

Disparity Index per site is shown for all sequence pairs. Values greater than 0 indicate the larger differences in base composition biases than expected based on evolutionary divergence between sequences and by chance alone. This analysis involved 6 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 674 positions (**Table 11**) in the final dataset. Evolutionary analyses were conducted in MEGA X.

5.5.6 Nucleotide Frequencies in COI sequences of *O. longicollis* geographical populations and banana cultivars

Nucleotide composition analysis in COI genes suggested that the genes were AT-rich in all the 20 geographical populations of *O. longicollis* and 6 geographical populations of scarring beetle. A significant ($P<0.05$) difference was observed in nucleotide composition among 20 geographical populations of *O. longicollis* and 9 different cultivars of banana (**Table 12**). Thymine (39.06%- pyrimidine) base occurred Most likely in substitution pattern followed by adenine (30.51%), cytosine (16.38%) and finally by guanine (14.05%). Highest nucleotide frequencies (T-39.16%) were observed in the following geographical populations of *O. longicollis* and (banana cultivars):

1) Tanhril (Vaibalhla, Balhlasen),
2) Theiriat (vaibalhla, Balhlakawl, Zobalhla, Kawlbalhla),
3) Darzo (Khumtungbalhla),
4) Cheural (Kawlbalh

2) Nucleotide composition analysis in CO genes suggested that the genes were AT-rich in all the 20 geographical populations of O. longicollis. A significant ($P<0.05$) difference was observed in nucleotide composition among 20

geographical populations of *O. longicollis* and 9 different cultivars of banana. Thymine (39.06%- pyrimidine) base occurred Most likely in substitution pattern followed by adenine (30.51%), cytosine (16.38%) and finally by guanine (14.05%). Highest nucleotide frequencies (T-39.16%) were observed in the following geographical populations of *O. longicollis* and (banana cultivars) (**Table 12**).

Table. 12. Nucleotide frequency in COI gene of *Odoiporus longicollis*.

	T(U)	C	A	G	Total	T-1	C-1	A-1	G-1	Pos #1	T-2	C-2	A-2	G-2	Pos #2	T-3	C-3	A-3	G-3	Pos #3
AZ SKM Changpui	39.04	16.41	30.54	14.01	835.00	30.47	16.13	29.03	24.37	279.00	41.73	22.30	20.14	15.83	278.00	44.96	10.79	42.45	1.80	278.00
AZ TH1 Vaibalhla	39.16	16.29	30.54	14.01	835.00	30.47	16.13	29.03	24.37	279.00	42.09	21.94	20.14	15.83	278.00	44.96	10.79	42.45	1.80	278.00
AZ TH2 Banria	39.16	16.29	30.54	14.01	835.00	30.47	16.13	29.03	24.37	279.00	42.09	21.94	20.14	15.83	278.00	44.96	10.79	42.45	1.80	278.00
AZ TH3 Banria	39.16	16.29	30.54	14.01	835.00	30.47	16.13	29.03	24.37	279.00	42.09	21.94	20.14	15.83	278.00	44.96	10.79	42.45	1.80	278.00
AZ TH4 Vaibalhla	38.92	16.41	30.54	14.13	835.00	30.11	16.13	29.03	24.73	279.00	42.09	21.94	20.14	15.83	278.00	44.60	11.15	42.45	1.80	278.00
AZ TH5 Banria	39.04	16.29	30.54	14.13	835.00	30.11	16.13	29.03	24.73	279.00	42.09	21.94	20.14	15.83	278.00	44.96	10.79	42.45	1.80	278.00
AZ TH6 Changthir	38.92	16.41	30.54	14.13	835.00	30.11	16.13	29.03	24.73	279.00	42.09	21.94	20.14	15.83	278.00	44.60	11.15	42.45	1.80	278.00
AZ TR Changpawl	39.04	16.29	30.54	14.13	835.00	30.11	16.13	29.03	24.73	279.00	42.09	21.94	20.14	15.83	278.00	44.96	10.79	42.45	1.80	278.00
LG TR1 Vaibalhla	39.16	16.29	30.54	14.01	835.00	30.47	16.13	29.03	24.37	279.00	42.09	21.94	20.14	15.83	278.00	44.96	10.79	42.45	1.80	278.00
LG TR2 Balhlakawl	39.16	16.29	30.54	14.01	835.00	30.47	16.13	29.03	24.37	279.00	42.09	21.94	20.14	15.83	278.00	44.96	10.79	42.45	1.80	278.00
LG TR3 Zobalhla	39.16	16.29	30.54	14.01	835.00	30.47	16.13	29.03	24.37	279.00	42.09	21.94	20.14	15.83	278.00	44.96	10.79	42.45	1.80	278.00
LG TP Kawlbalhla	39.16	16.29	30.54	14.01	835.00	30.47	16.13	29.03	24.37	279.00	42.09	21.94	20.14	15.83	278.00	44.96	10.79	42.45	1.80	278.00
LG DZ Khumtungbalhla	39.16	16.29	30.54	14.01	835.00	30.47	16.13	29.03	24.37	279.00	42.09	21.94	20.14	15.83	278.00	44.96	10.79	42.45	1.80	278.00

LG SV Balhlakawl	39.04	16.53	30.42	14.01	835.00	30.47	16.13	29.03	24.37	279.00	41.73	22.30	20.14	15.83	278.00	44.96	11.15	42.09	1.80	278.00
LNT SG1 Balhlakawl	38.80	16.65	30.42	14.13	835.00	30.11	16.49	29.03	24.37	279.00	41.73	22.30	20.14	15.83	278.00	44.60	11.15	42.09	2.16	278.00
LNT CR1 Vaibalhla	38.92	16.53	30.42	14.13	835.00	30.47	16.13	29.03	24.37	279.00	42.09	21.94	20.14	15.83	278.00	44.24	11.51	42.09	2.16	278.00
LNT CH2 Khumtungbalhla	39.16	16.29	30.42	14.13	835.00	30.47	16.13	28.67	24.73	279.00	42.09	21.94	20.14	15.83	278.00	44.96	10.79	42.45	1.80	278.00
LNT RL Vaibalhla	39.04	16.53	30.42	14.01	835.00	30.47	16.13	29.03	24.37	279.00	41.73	22.30	20.14	15.83	278.00	44.96	11.15	42.09	1.80	278.00
LNT SG2 Zobalhla	39.04	16.41	30.54	14.01	835.00	30.11	16.49	29.03	24.37	279.00	42.09	21.94	20.14	15.83	278.00	44.96	10.79	42.45	1.80	278.00
S SH Vaibalhla	38.92	16.53	30.66	13.89	835.00	29.75	16.85	29.03	24.37	279.00	42.09	21.94	20.14	15.83	278.00	44.96	10.79	42.81	1.44	278.00
Avg.	39.06	16.38	30.51	14.05	835.00	30.32	16.20	29.01	24.46	279.00	42.01	22.01	20.14	15.83	278.00	44.87	10.92	42.39	1.82	278.00

Nucleotide composition analysis in CO genes suggested that the genes were AT-rich in all the 6 geographical populations of scarring beetle. A significant ($P<0.05$) difference was observed in nucleotide composition among 6 geographical populations of *O. longicollis* and 9 different cultivars of banana (**Table 13**). Thymine (31.56%- pyrimidine) base occurred Most likely in substitution pattern followed by adenine (33.84%), cytosine (15.89%) and finally by guanine (18.71%). Highest nucleotide frequencies (T-33.15%) were observed in the following geographical populations of scarring beetle and (banana cultivars).

Table. 13. Nucleotide frequency in COI gene of *B. subcostatum*.

	MZ AZ SITE1	MZ AZ SITE2	MZ AZ SITE3	MZ AZ SITE4	MZ AZ SITE5	MZ AZ SITE6	Avg.
T(U)	31.351	31.171	31.171	31.171	31.351	33.153	31.562
C	15.135	15.315	15.315	15.315	14.955	19.279	15.886
A	34.054	34.054	34.054	34.054	34.234	32.613	33.844
G	19.459	19.459	19.459	19.459	19.459	14.955	18.709
Total	555.000	555.000	555.000	555.000	555.000	555.000	555.00
T-1	16.216	16.216	16.216	16.216	16.216	20.219	16.877
C-1	16.216	16.216	16.216	16.216	16.216	19.126	16.697
A-1	41.622	41.622	41.622	41.622	41.622	34.426	40.433
G-1	25.946	25.946	25.946	25.946	25.946	26.230	25.993
Pos #1	185	185	185	185	185	183	184.66
T-2	32.973	32.973	32.973	32.973	32.973	39.459	34.054
C-2	26.486	26.486	26.486	26.486	26.486	25.946	26.396
A-2	21.081	21.081	21.081	21.081	21.081	17.838	20.541
G-2	19.459	19.459	19.459	19.459	19.459	16.757	19.009
Pos #2	185	185	185	185	185	185	185
T-3	44.865	44.324	44.324	44.324	44.865	39.572	43.705
C-3	2.703	3.243	3.243	3.243	2.162	12.834	4.586
A-3	39.459	39.459	39.459	39.459	40.000	45.455	40.558
G-3	12.973	12.973	12.973	12.973	12.973	2.139	11.151
Pos #3	185	185	185	185	185	187	185.33

5.5.7 Relative synonymous codon usage statistic (RSCU)

All frequencies are averages over all taxa. Relative synonymous codon usage (RSCU) is given in parentheses following the codon frequency.

In this study, we analysed compositional features and codon usage of MT-COI gene among the 20 geographical populations of *O. longicollis* to explore the pattern of codon usage bias infesting 9 banana cultivars. Average number of codons usage was 278(**Table 14).** Codon usage bias was observed in 19 codons.

In this study, we analysed compositional features and codon usage of MT-COI gene among the six geographical populations of *B. subcostatum* to explore the pattern of codon usage bias infesting 9 banana cultivars. Average number of codons usage was 176 (**Table 15**). Codon usage bias was observed in 17 codons.

Table. 14. Codon usage Relative synonymous codon usage (RSCU) in COI sequences of *Odoiporus longicollis* geographical populations and banana cultivars.

UUU(F)	**23.6(1.76)**	UCU(S)	**8.1(2.22)**	UAU(Y)	**12.0(1.71)**	UGU(C)	0.0(0.00)
UUC(F)	3.3(0.24)	UCC(S)	2.1(0.58)	UAC(Y)	2.0(0.29)	UGC(C)	0.0(0.00)
UUA(L)	**17.0(3.39)**	UCA(S)	**7.6(2.10)**	UAA(*)	0.0(0.00)	UGA(*)	**8.0(3.00)**
UUG(L)	0.0(0.00)	UCG(S)	0.0(0.00)	UAG(*)	0.0(0.00)	UGG(W)	0.0(0.00)
CUU(L)	5.1(1.02)	CCU(P)	**6.0(2.14)**	CAU(H)	**9.0(1.80)**	CGU(R)	1.0(0.67)
CUC(L)	1.0(0.20)	CCC(P)	0.1(0.02)	CAC(H)	1.0(0.20)	CGC(R)	0.0(0.00)
CUA(L)	7.0(1.39)	CCA(P)	**5.1(1.82)**	CAA(Q)	**5.0(1.67)**	CGA(R)	**3.0(2.00)**
CUG(L)	0.1(0.01)	CCG(P)	0.1(0.02)	CAG(Q)	1.0(0.33)	CGG(R)	1.0(0.67)
AUU(I)	**22.9(1.53)**	ACU(T)	**11.2(2.94)**	AAU(N)	4.0(1.14)	AGU(S)	3.0(0.83)
AUC(I)	7.9(0.53)	ACC(T)	0.0(0.00)	AAC(N)	3.0(0.86)	AGC(S)	1.0(0.28)
AUA(I)	14.0(0.94)	ACA(T)	4.0(1.06)	AAA(K)	**5.1(1.68)**	AGA(R)	**4.0(2.67)**
AUG(M)	0.0(0.00)	ACG(T)	0.0(0.00)	AAG(K)	1.0(0.32)	AGG(R)	0.0(0.00)
GUU(V)	2.1(0.54)	GCU(A)	**7.0(1.62)**	GAU(D)	5.0(1.43)	GGU(G)	5.0(0.87)
GUC(V)	2.0(0.53)	GCC(A)	3.1(0.71)	GAC(D)	2.0(0.57)	GGC(G)	2.0(0.35)
GUA(V)	11.0(2.92)	GCA(A)	**7.2(1.67)**	GAA(E)	**6.0(2.00)**	GGA(G)	**14.0(2.43)**
GUG(V)	0.0(0.00)	GCG(A)	0.0(0.00)	GAG(E)	0.0(0.00)	GGG(G)	2.0(0.35)

Table. 15. RSCU for *Basilepta subcostatum*.

Codon	RSCU	Codon	RSCU	Codon	RSCU	Codon	RSCU
UUU(F)	1.42	UCU(S)	**1.91**	UAU(Y)	2	UGU(C)	2
UUC(F)	0.58	UCC(S)	0.14	UAC(Y)	0	UGC(C)	0
UUA(L)	**1.96**	UCA(S)	1.41	UAA(*)	1.26	UGA(*)	1.33
UUG(L)	0	UCG(S)	0.35	UAG(*)	0.42	UGG(W)	1
CUU(L)	1.35	CCU(P)	**1.82**	CAU(H)	**1.56**	CGU(R)	0.48
CUC(L)	0.13	CCC(P)	0.42	CAC(H)	0.44	CGC(R)	0
CUA(L)	**2.22**	CCA(P)	1.25	CAA(Q)	0.75	CGA(R)	0.96
CUG(L)	0.34	CCG(P)	0.52	CAG(Q)	1.25	CGG(R)	0
AUU(I)	**1.85**	ACU(T)	**1.67**	AAU(N)	**1.84**	AGU(S)	**1.84**
AUC(I)	0	ACC(T)	0.39	AAC(N)	0.16	AGC(S)	0.35
AUA(I)	1.15	ACA(T)	**1.94**	AAA(K)	**1.82**	AGA(R)	**2.4**
AUG(M)	1	ACG(T)	0	AAG(K)	0.18	AGG(R)	**2.16**
GUU(V)	**2.33**	GCU(A)	**2.41**	GAU(D)	**1.76**	GGU(G)	**1.89**
GUC(V)	0.06	GCC(A)	0.1	GAC(D)	0.24	GGC(G)	0
GUA(V)	1.31	GCA(A)	1.25	GAA(E)	1.35	GGA(G)	1
GUG(V)	0.3	GCG(A)	0.24	GAG(E)	0.65	GGG(G)	1.11

5.5.8 Estimating Synonymous and Nonsynonymous Substitution Rates Under Realistic Evolutionary Models

Mean values of the nucleotide substitution matrices were as follows:

Synonymous substitutions ds= 0.0073, Nonsynonymous substitutions dn= 0.0026

ds/dn ratio= 3.109, ps/pn ratio= 3.097, total number of mutations observed was 47.

Average mutations per sequence notified as transition- 2.2 and transversion- 0.3.

Table. 16. Estimating Synonymous and Nonsynonymous Substitution Rates Under Realistic Evolutionary Model for *O. longicollis*.

Sequence names	S	N	ps	pn	ds	dn	ds/dn	ps/pn
MZ_AZ_TH1_ Vaibalhla	196.67	637.3333	<0.0051	0.0016	<0.0051	0.0016	<3.2483	<3.2407
MZ_AZ_TH2_ Banria	196.67	637.3333	<0.0051	0.0016	<0.0051	0.0016	<3.2483	<3.2407
MZ_AZ_TH3_ Banria	196.67	637.3333	<0.0051	0.0016	<0.0051	0.0016	<3.2483	<3.2407
MZ_AZ_TH4_ Vaibalhla	196.67	637.3333	0.0051	0.0031	0.0051	0.0031	1.6225	1.6203
MZ_AZ_TH5_ Banria	196.67	637.3333	<0.0051	0.0031	<0.0051	0.0031	<1.6225	<1.6203
MZ_AZ_TH6_ Changthir	196.67	637.3333	0.0051	0.0031	0.0051	0.0031	1.6225	1.6203
MZ_AZ_TR_ Changpawl	196.67	637.3333	<0.0051	0.0031	<0.0051	0.0031	<1.6225	<1.6203
MZ_LG_TR1_ Vaibalhla	196.67	637.3333	<0.0051	0.0016	<0.0051	0.0016	<3.2483	<3.2407
MZ_LG_TR2_ Balhlakawl	196.67	637.3333	<0.0051	0.0016	<0.0051	0.0016	<3.2483	<3.2407
MZ_LG_TR3_ Zobalhla	196.67	637.3333	<0.0051	0.0016	<0.0051	0.0016	<3.2483	<3.2407
MZ_LG_TP_ Kawlbalhla	196.67	637.3333	<0.0051	0.0016	<0.0051	0.0016	<3.2483	<3.2407
LG_DZ_ Khumtungbalhla	196.67	637.3333	<0.0051	0.0016	<0.0051	0.0016	<3.2483	<3.2407
MZ_LG_SV_ Balhlakawl	196.84	637.1667	0.0102	0.0031	0.0102	0.0031	3.2524	3.2371
LNT_SG1_ Balhlakawl	197.17	636.8333	0.0101	0.0047	0.0102	0.0047	2.1612	2.1533
MZ_LNT_CR1_ Vaibalhla	196.67	637.3333	0.0153	0.0016	0.0154	0.0016	9.812	9.722
MZ_LNT_CH2_ Khumtungbalhla	196.84	637.1667	<0.0051	0.0031	<0.0051	0.0031	<1.6206	<1.6185
MZ_LNT_RL_ Vaibalhla	196.84	637.1667	0.0102	0.0031	0.0102	0.0031	3.2524	3.2371
MZ_LNT_SG2_ Zobalhla	196.67	637.3333	0.0102	0.0031	0.0102	0.0031	3.256	3.2407
MZ_S_SH_ Vaibalhla	197	637	0.0152	0.0047	0.0154	0.0047	3.2565	3.2335

COI mitochondrial molecular marker identified four main haplogroups from **Table-17** as: Group A- 7 populations (Vanlalphal, Rawlbawk, Sangua, Cherual and Saiha), Group B- 5 populations (Skawrtchhuichhu and Tanhril), Group C- 6 populations (Tanhril, Theiriat, Darzo), Group D- 2 populations (Theiriat and Tuipui).

The **Table 17** clearly showed four haplogroups Group A: Site-1 Aizawl and Site-5 Aizawl, Group B: Site-6 Aizawl, Group C: Site-2 and 3 Aizawl, and Group D: Site-4 Aizawl.

Table. 17. Estimating Synonymous and Nonsynonymous Substitution Rates Under Realistic Evolutionary Model for *B. subcostatum*.

Compare	Sequence names	S	N	ps	pn	ds	dn	ds/dn	ps/pn
0 1	Aizawl SITE2	129.34	398.67	0.0077	<0.0025	<0.0078	<0.0025	>3.0933	>3.0825
0 2	Aizawl SITE3	129.34	398. 67	0.0077	<0.0025	<0.0078	<0.0025	>3.0933	>3.0825
0 3	Aizawl SITE4	129.34	398. 67	0.0077	<0.0025	<0.0078	<0.0025	>3.0933	>3.0825
0 4	Aizawl SITE5	129.34	398. 67	0.0077	<0.0025	<0.0078	<0.0025	>3.0933	>3.0825
0 5	Aizawl SITE6	94	287	0.406	0.3618	0.5846	0.4939	1.1837	1.1223

5.5.9 Cumulative dS/dN graph *Odoiporus longicollis* and *Basilepta subcostatum*

Synonymous and nonsynonymous substitution rates were calculated using Highlighter and SNAP- HIV data base tools (Yang and Nielsen, 2000) using the link (www.hiv.lanl.gov/content/sequence/HIV/HIVTools.html).

In **Figure 25** the green colour showing synonymous, red colour showing nonsynonymous, blue colour showing indels and grey colour showing stops. This graph shows that nonsynonymous substitution more compare to synonymous substitution i.e. significant, so it clearly tells that mutation is happening between and within the population.

The **Figure 26** clearly shows that nonsynonymous substitution more compare to synonymous substitution i.e. significant, so it clearly tells that mutation is happening between and within the population.

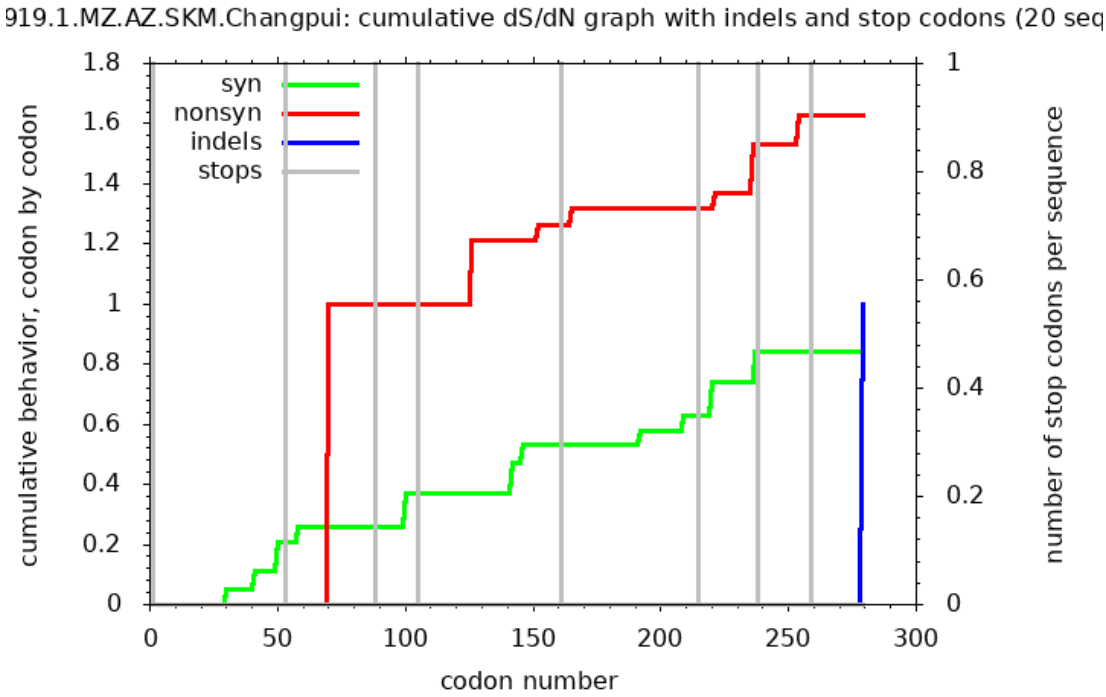

Fig. 27. Cumulative dS/dN graph of *O. longicollis*, where the blue, red and green colour lines represents indels, nonsynonymous and synonymous substitution respectively.

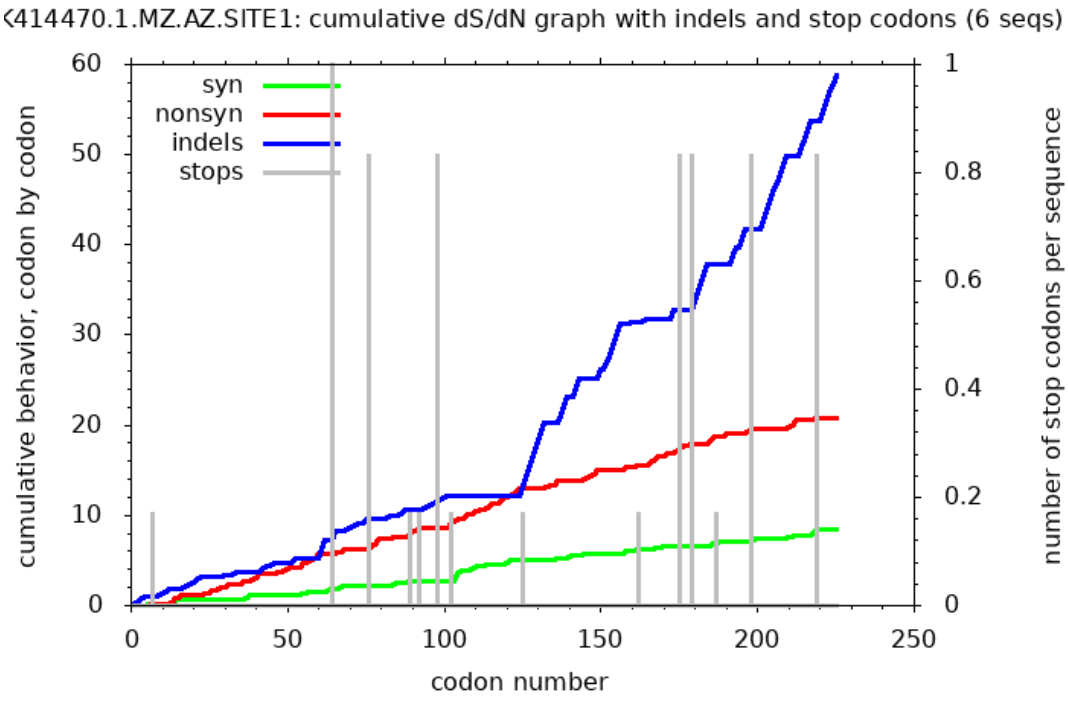

Fig. 28. Cumulative dS/dN graph of *B.subcostatum*, where the blue, red and green colour lines represents indels, nonsynonymous and synonymous substitution respectively.

5.14 Tajima's Neutrality Test

Table. 18. Tajima's Neutrality Test *Odoiporus longicollis*.

m	S	ps	Θ	π	D
20	**18**	**0.021557**	**0.006076**	**0.003284**	**-1.72888**

Note: In this figure m = number of sequences, n = total number of sites, S = Number of segregating sites, ps = S/n, Θ = ps/a1, π = nucleotide diversity, and D is the Tajima test statistic.

This analysis involved 20 nucleotide sequences (**Table 18**). Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 835 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

Table. 19. Tajima's Neutrality Test *Basilepta subcostatum*.

m	S	ps	Θ	π	D
6	159	0.235905	0.103316	0.078932	-1.530426

This analysis involved 6 nucleotide sequences (**Table 19**). Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 674 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

5.5.11 Evolutionary analysis for geographical population of *O. longicollis* and *B. subcostatum* by Maximum Likelihood method.

It is the best tree found based on assumptions on evolution model. Nucleotide models more advanced at the moment than amino acid models. Programs require lot of capacity from the system.

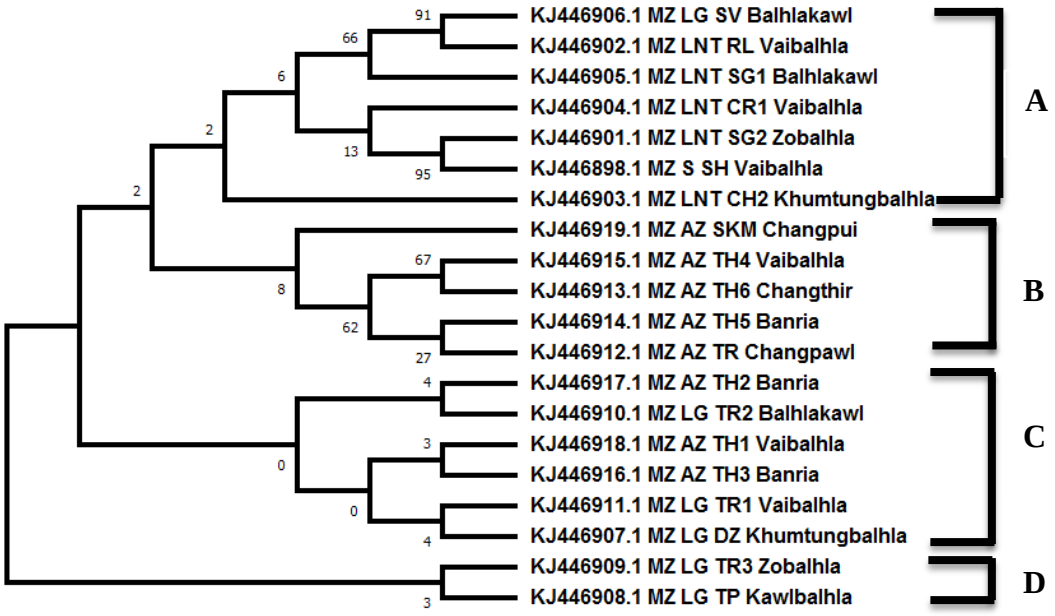

Fig. 29. Maximum Likelihood Phylogenetic tree of *O. longicollis* of COI gene of twenty geographical populations having four haplotypes (A, B, C and D) based on the Tamura 3 parameter model.

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (**Figure 27**). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. This analysis involved 20 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 835 positions in the final dataset. Evolutionary analyses were conducted in MEGA X. **Figure 27** clearly showed four haplogroups Group A- 7 populations (Vanlalphal, Rawlbawk, Sangua, Cherual and Saiha), Group B- 5 populations (Skawrtchhuichhu and Tanhril), Group C- 6 populations (Tanhril, Theiriat, Darzo), Group D- 2 populations (Theiriat and Tuipui).

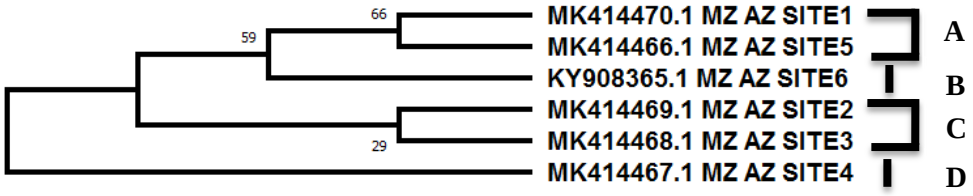

Fig. 30. Maximum Likelihood Phylogenetic tree of *B. subcostatum* of COI gene of six geographical populations having four haplotypes based on the Tamura 3 parameter model.

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model, 1993. The tree with the highest log likelihood (-1363.20) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches (**Figure 28**). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix

of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 6 nucleotide sequences. There were a total of 674 positions in the final dataset. Evolutionary analyses were conducted in MEGA X. **Figure 28** clearly showed four haplogroups Group A: Site-1 Aizawl and Site-5 Aizawl, Group B: Site-6 Aizawl, Group C: Site-2 and 3 Aizawl, and Group D: Site-4 Aizawl.

CHAPTER 6

DISCUSSION

DISCUSSION

A full account of life cycle of *O. longicollis* studies has been recognized by Isahaque, (1978), Visalakshi *et al*, (1989), Padmanabhan, (2001), Tiwari *et.al*, (2006), and Thippaiah *et al*, (2011). Life span determined in the current study for the grub from instar 1 to 4th is in support with the findings of Thippaiah *et al*, (2011). The pre-pupal and pupal stages together showed on an average of 10-14 days, and this finding is in agreement with the observations of Visalakshi *et al*, (1989) and *et al*, (2014), while Thippaiah *et al*, (2011) record it as 17-21 days. Total developmental period of BPW in the current investigation was in agreement with the findings of Visalakshi *et al*, (1989) and Thippaiah *et al*. (2011), (2014), Pre oviposition period was 23-28 days and this was in the range previously recorded by Visalakshi *et al*, (1989), and Thippaiah *et al*,(2011) in our study we found 20-30 days which matched with Visalakshi *et al*, (1989) and Thippaiah *et al*, (2011).

Difference in the longevity of *O. longicollis* has been reported. Longevity in the present investigation recorded 180 days, and it is meticulously in agreement with the observation of Ravi and Palaniswami, (2002), they report 200 days. However, Pinto (1928) examined it as high as 2 years, but Padmanabhan and Sathiamoorthy, (2001), stated it was only 44 and 81.0 days, respectively. Thippaiah *et al*, (2011) studied longevity of both male and female under known temperature and observed, irrespective of sex, the average longevity was 75-80 days.

The active and sustainable administration of pests mainly relies with the policies followed for the performances of integrated pest management (IPM). Therefore, development and application of IPM involves a systematic investigation of the population structure, dynamics and phylogeography of the planned pest species as they generally deviate from region to region. The studies of the genetic dissimilarities in insect pest populations have been enormously useful in providing evidence on histories of migration configuration and population statistics (Roderick, 1996; Avise, 2000).

The tools intricate for investigative the source and distribution of a species are consequently, widely known as a good monitoring system (Zhang *et al*., 2010).

There are only few earlier studies detailing the genetic structure of population and biogeography with the provision of molecular markers of this economically important insect pest species, *O. longicollis* (Marimuthu *et al*., 2009).

However, lacking data on species diversity, with orientation to tools on molecular markers, especially, the information related to nucleotide sequences on the population structure; prevent the development of IPM approaches for the organizing banana pseudostem borer, *O. longicollis*. The study on the bioecology of population in many organisms somewhat recently involved the use of genes in the mitochondrial genome (Hebert *et al*., 2003; Caragiulo *et al*., 2014). Understanding the sequence features of the COI provided better understanding into the population genetic structure (Liu *et al*., 2013).

The average nucleotide composition of *O. longicollis* COI region between various populations showed an average AT content of 69.56 % and GC content of 30.44 %. This is relating with AT rich nature of COI gene sequences described previously (Simons *et al*., 1994; Chang *et al*., 2014). The negative values showed by Tajima's D tests indicate population size expansion (e.g., after a bottleneck or a selective sweep) and/or purifying selection (Tajima, 1989). There were four haplotypes identified with a haplotype diversity of 0.95, out of 20 different populations of *O. longicollis* and 0.91out of 6 six populations of scarring beetle samples collected from four district of Mizoram suggesting insignificant independent histories of distribution and gene flow (Chang *et al*., 2014).

There were four main clusters of mitochondrial genome of *O. longicollis* was separated by the phylogenetic construction based on COI sequences. It is amazing to mention that the group-A with seven populations, viz., Lunglei South Vanlalphal Balhlakawl, Lawntlei Rawlbuk Vaibalhla, Lawntlei Sangua 1 Balhlakawl, Lawntlei Cheural1 Vaibalhla, Saiha Vaibalhla, Lawntlei Cheural 2 Khumtungbalhla, Lawntlei Cherual1 Zobalhla in one mono-phyletic group. Likewise, the populations of Aizawl Sakawrchhuichhum Changpui, Aizawl Tanhril 4 Vaibalhla, Aizawl Tanhril 6 Changthir, Aizawl Tanhril 5 Banria, Aizawl Tuirial Changpui formed the second group-B and Aizawl Tanhril 2 Banria, Lunglei Theiriat2 Balhlakawl, Aizawl

Tanhril1 Vaibalhla, Aizawl Tanhril 3 Banria, Lunglei Theiriat Vaibalhla, Lunglei Darzo Khumtungbalhla in group-C and Lunglei Theiriat Zobalhla, Lunglei Tuipui Kawlbalhla in group-D (Chang *et al*., 2014).

The haplotype network study in the current study revealed four different clades among 20 populations of *O. longicollis* and scarring beetle collected at different regions of Mizoram that was matching almost with the phylogenetic structure, showing distribution of a few genetic variation as a result of geographical locations and also revealed by neutrality test and mismatch distribution analysis (Servedio and Noor, 2003)

CHAPTER 7

SUMMARY

- Nine different banana cultivars (Banria, Balhlasen, Changthir, Changpawl, Changpui, Khumtungbalhla, Lawngbalhla, Zobalhla and Vaibalhla) were identified and characterized by using standard protocols and performed the bioecological studies of *O. longicollis* and *B. subcostatum* in twenty sites of four districts of Mizoram.
- The time period observed from egg to the adult banana pseudostem weevil was found to be 46 ± 7.2 (35 to 58) days in summer (May to August) and 64 ± 11.4 (47 to 85) days in winter (November to February).
- Population dynamics study of *O. longicollis* discovered that in rainy season with high temperature reduced the infestation rate along with decreased population size. The population size and infestation pattern rate of the *O. longicollis* were negatively correlated with meteorological parameters.
- The infestation pattern rate and population distribution of *B. subcostatum* increased during summer season. The population and infestation rate was maximum in the month of August and lowest in December. The population size and infestation of the *B. subcostatum* were positively correlated with meteorological parameters.
- Infestation pattern and population distribution (larva, pupa and adult) of *O. longicollis* and *B. subcostatum* were detected to be high in *Musa balbisiana* (Changthir) followed by *Musa acuminata* (Changpui), *Musa paradisiaca* (Changpawl), in the chosen locations of four districts of Mizoram.
- The randomly amplified polymorphic DNA (RAPD) marker study displayed no amplification results with 19 primers both in *O. longicollis* and *B. subcostatum*.
- Because of no results found in RAPD analysis as an alternative COI marker was chosen for population genetic structure analysis of *O. longicollis* and *B. subcostatum*.
- Based on low BIC, AIC and InL scores the Tamura 3 parameter model was assessed as the finest model for COI gene sequence of both *O.*

longicollis and *B. subcostatum*. Gamma parameter value i.e. site rate variation, was found to be high in A and T than C and G.

- In *O. longicollis*, it was found that 817 conserved site, 18 variable sites, and 8 parsimony informative sites, 10 singleton sites out of 835 nucleotides of the CO1 gene and in case of *B. subcostatum*, 396 conserved sites, variables sites 159, 1 parsimony informative sites, 158 singleton sites out of 674 nucleotides.
- Estimation of base composition bias difference between twenty sequences in *O. longicollis* and six in *B. subcostatum* showed violation of the assumption of equality of substitution rates between geographical population and banana cultivars and substitution patterns are not homogenous ($P< 0.05$).
- The subsequent nucleotide composition analysis in COI was estimated that the frequency of AT was higher than the GC in all the twenty geographical population of *O. longicollis* and six geographical population of *B. subcostatum*.
- RSCU statistical analysis revealed that the codon usage bias was observed in nineteen codons out of 278 in COI gene of *O. longicollis* and seventeen codons out of 176 in COI gene of *B. subcostatum*.
- The average COI haplotype diversity ($h = 0.95$) in *O. longicollis* and ($h = 0.91$) in *B. subcostatum* specifies the effectiveness of COI sequence variation in detecting genetic structure.
- The nucleotide and amino acid variability of COI confirmed that the ratio of nonsynonymous to synonymous substitutions is high, indicating that COI gene is subject to strong positive selection.
- Estimation of synonymous and nonsynonymous substitution rates under realistic evolutionary models nonsynonymous is high in COI gene of *B. subcostatum* indicates a positive selection.
- Negative Tajima's (D) indicates an excess of low frequency polymorphisms relative to expectation, indicating population size expansion and/or purifying selection in both *O. longicollis* and *B. subcostatum*.

- COI mitochondrial molecular marker identified four main haplogroups in twenty geographical populations of *O. longicollis* and six geographical populations of *B. subcostatum* respectively.
- Based on the population genetic structure analysis (Model test, transition/transversion bias, codon usage, substitution matrix, Tajima test of neutrality and phylogenetic trees) of COI sequence of *O. longicollis* and *B. subcostatum* revealed the fact that the effect of geographic isolation on genetic structure is the model of isolation by distance (IBD), which forecasts that genetic differentiation between twenty geographical populations and six geographical populations increases with geographic distances (Aizawl, Lunglei, Saiha and Lawngtlei).
- Further it is proved that there is a strong correlation between genetic variation in banana cultivars and host associated differentiation in COI genetic makeup of *O. longicollis* and *B. subcostatum*.

CHAPTER 8

APPENDIX

List of acronyms

Abbreviated	Full form
o	Degree
%	Per cent
µl	Micro litre
cm	Centimetre
h	Hour
Min	Minute
PCR	Polymerase chain reaction
DNA	Deoxyribonucleic acid
RAPD	Random amplifying polymorphic DNA
mM	Milimolar
BSA	Bovine serum albumin
dNTP	Deoxyribonucleotide triphosphate
$MgCl_2$	Magnesium chloride
pmol	Pico mole
sec	Second
µg/ml	Micro gram per mili litre
TBE	Tris-borate-EDTA
EDTA	Ethylenediamine tetraacetic acid
ng	Nano gram
mm	Milimeter
COI	Cytochrome oxydase I
MEGA	Molecular evolutionary genetics analysis
RSCU	Relative synonymous codon usage

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D.O.B : 23.03.1994

TEMPORARY ADDRESS: Department of Zoology, Mizoram University, Aizawl

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Via- Bangriosi, Dist- Mayurbhanj
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EDUCATION QUALIFICATION:

SN	Name of Exam	Board/university	Year of Passing	Percentage/CGPA
1	10^{th}	B.S.E	2009	57%
2	+2	C.H.S.C	2011	53%
3	+3 (Botany)	N.O.U	2014	61.5%
4	P.G (Biotechnology)	N.O.U	2017	7.13

Particulars of the candidate

Name of the candidate	: ABINASH GIRI
Degree	: Master in Philosophy
Department	: Zoology
Title of dissertation	: Population and genetic diversity in banana pseudostem weevil and leaf and fruit scarring beetle, inferred from RAPD fingerprints
Date of admission	: 01.08.2019
Commencement of dissertation	: January 2020 to April 2021
Approval of research proposal	
1. BOS	: 02.06.2020
2. School board	: 12.06.2020
Registration No. & date	: MZU/M. Phil. /590 of 12.06.2020
Due date of submission	: December
Extension	: 02.06.2021

HEAD

Department of Zoology

विभागाध्यक्ष
Head
जीवविज्ञानविभाग
Department of Zoology
मिजोरम विश्वविद्यालय
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मिजोरम विश्वविद्यालय
आईजल-७९६००४
भारतीय संसद द्वारा पारित एक्ट के अन्तर्गत स्थापित एक केन्द्रिय विश्वविद्यालय
MIZORAM UNIVERSITY
Aizawl – 796004
A Central University Established by Parliament Act No.8 of 2000 (25.4.2000)
NAAC Accredited Grade 'A' University

No.16-2/MZU(Acad)/20/394-399 Dated. 11th February 2021

NOTIFICATION

Consequent upon Resolution No. **AC:39:4(4)**, this is to notify that the 39th Meeting of the Academic Council held on 20.11.2020, resolved to approve **Extension of M.Phil. Registration Period** submitted by the respective Board of schools as under:

1. The **School Board of Economics, Management & Information Science** (05.11.2020)

Sl No	Department	Name of Scholars	Date of Admission	Registration No.	Extension Period Date/Month/Year	Name of Supervisor / Joint Supervisor
1	Economics	B. Lalchhandama	28.08.2019	A/F	One Semester (up to31.07.2021)	Dr. K. Angela Lalhmingsangi
2	Economics	C. Sangthuamluaia	27.08.2019	A/F	One Semester (up to31.07.2021)	Dr.James L.T. Thanga
3	Economics	P.C. Remlalhruaii	27.08.2019	A/F	One Semester (up to31.07.2021)	Dr. Lalhriatpuii
4	Mass Communication	Gospel Lalawmpuii	18.07.2019	MZU/M.Phil./566 Dt.29.05.2020	One Semester (up to31.07.2021)	Dr. Lalremruati Khiangte
5	Mass Communication	Vanlalhlimpuia	25.07.2019	MZU/M.Phil./567 Dt.29.05.2020	One Semester (up to31.07.2021)	Dr. Irene Lalruatkimi
6	Management	V L Nuntluanga	30th July 2019	MZU/M.Phil./565 of 29.05.2020	One Semester (up to31.07.2021)	Prof. L. S. Sharma
7	Management	Alexius Lalchhandama	30th July 2019	MZU/M.Phil/564 of 29.05.2020	One Semester (up to31.07.2021)	Dr Lalhminglian a Renthlei
8	Management	K.Lalramnghaka	30th July 2019	A/F	One Semester (up to31.07.2021)	Dr. Bidhu. Kanti Das
9	Commerce	Brian Laldinsanga	27/07/2019	MZU/M.Phil./558 of 29.05.2020	One Semester (up to31.07.2021)	Prof. NVR Jyoti Kumar
10	Commerce	Juti Moni Barman	24/07/2019	A/F	One Semester (up to31.07.2021)	Prof. Bhartendu Singh
11	Commerce	Lallawmzuali Chhakchhuak	25.07.2019	MZU/M.Phil./610 of 29.05.2020	One Semester (up to31.07.2021)	Dr. Lalneihtluangi Fanai
12	Library & Information Science	Lalhmangaihsangi Sailo	24.07.2019	MZU/M.Phil./561 of 29.05.2020	One Semester (up to31.07.2021)	Prof. Pravakar Rath

Contd…2

Sl No	Department	Name of Scholars	Date of Admission	Registration No.	Extension Period Date/Month/Year	Name of Supervisor / Joint Supervisor
10	Histor & Ethnography	Lalmalsawmi Thadou	23.07.2019	MZU/M.Phil/607 of 12.06.2020	31.07.2021	Dr.Hmingthan-zuali
11	Histor & Ethnography	S. Lalthlamuan Vaiphei	18.07.2019	MZU/M.Phil/608 of 12.06.2020	31.07.2021	Prof. Lalngur-liana Sailo
12	Histor & Ethnography	Laura Pulamate	24.07.2019	MZU/M.Phil/619 of 12.06.2020	31.07.2021	Prof. Lalngu-rliana Sailo
13	Social Work	V. Lalramchuani	19.8.2019	MZU/M.PHIL/613 of 12.06.2020	31.07.2020	Prof. Kanagaraj Easwaran Jt. Supervisor: Prof. C. Devendiran
14	Social Work	Michael Vanromawia	19.8.2019	MZU/M.PHIL/617 of 12.06.2020	31.07.2020	Dr. C. Lalengzama
15	Social Work	Lalrinzuala	19.8.2019	MZU/M.PHIL/615 of 12.06.2020	31.07.2020	Dr. H. Elizabeth
16	Social Work	Lalduhsanga Sailo	19.8.2019	MZU/M.PHIL/616 of 12.06.2020	31.07.2020	Dr. Grace Lalhlupuii Sailo
17	Social Work	Zohmunpuia	20.8.2019	MZU/M.PHIL/614 of 12.06.2020	31.07.2020	Prof. C. Devendiran
18	Social Work	Diana Lalrinsiami Chhakchhuak	20.8.2019	MZU/M.PHIL/598 of 12.06.2020	31.07.2020	Dr. Henry Zodinliana Pachuau
19	Social Work	Ngurhlunchhungi	19.8.2019	A/F	31.07.2020	Prof. C. Devendiran

V. The School Board of Life Sciences (04.11.2020)

Sl No	Department	Name of Scholars	Date of Admission	Registration No.	Extension Period Date/Month/Year	Name of Supervisor / Joint Supervisor
1	Zoology	Abinash Giri	01.08.2019	MZU/M.Phil/590 of 12.06.2020	**02.06.2021**	Prof. G. Gurusubramanian
2	Zoology	Pori Buragohain	02.08.2019	MZU/M.Phil/591 of 12.06.2020	**01.06.2021**	Prof. G. Gurusubramanian
3	Biotechnology	Maisnam Akbar Singh	06.08.2019	A/F	**05.06.2021**	Dr. Th. Robert Singh

Contd…6

LIST OF CONFERENCE/SEMIINAR/WORKSHOP/WEBINAR ATTEND AND PARTICIPITE

Sl No.	Conference/Seminar/Workshop/Webinar	Date
1	National Workshop on Bioinformatics for Zoologist	26th -31st August 2019
2	National Workshop on Ethics in Research and Preventing Plagiarism (ERPP 2019)	3rd October 2019
3	International conference on advances in animal sciences (ICRAAS-2019)	6th – 8th November 2019
4	Webinar on Antibiotic Resistance	17th July 2020
5	Online Workshop on Sequence to database and database to sequence	6th – 12th January 2021

BIOINFORMATICS INFRASTRUCTURE FACILITY (BIF)
DEPARTMENT OF BIOTECHNOLOGY
Mizoram University
(Accredited with 'A' Grade by NAAC)
Aizawl – 796004

Certificate

Certified that Abinash Giri *Participated / ~~acted as a~~*

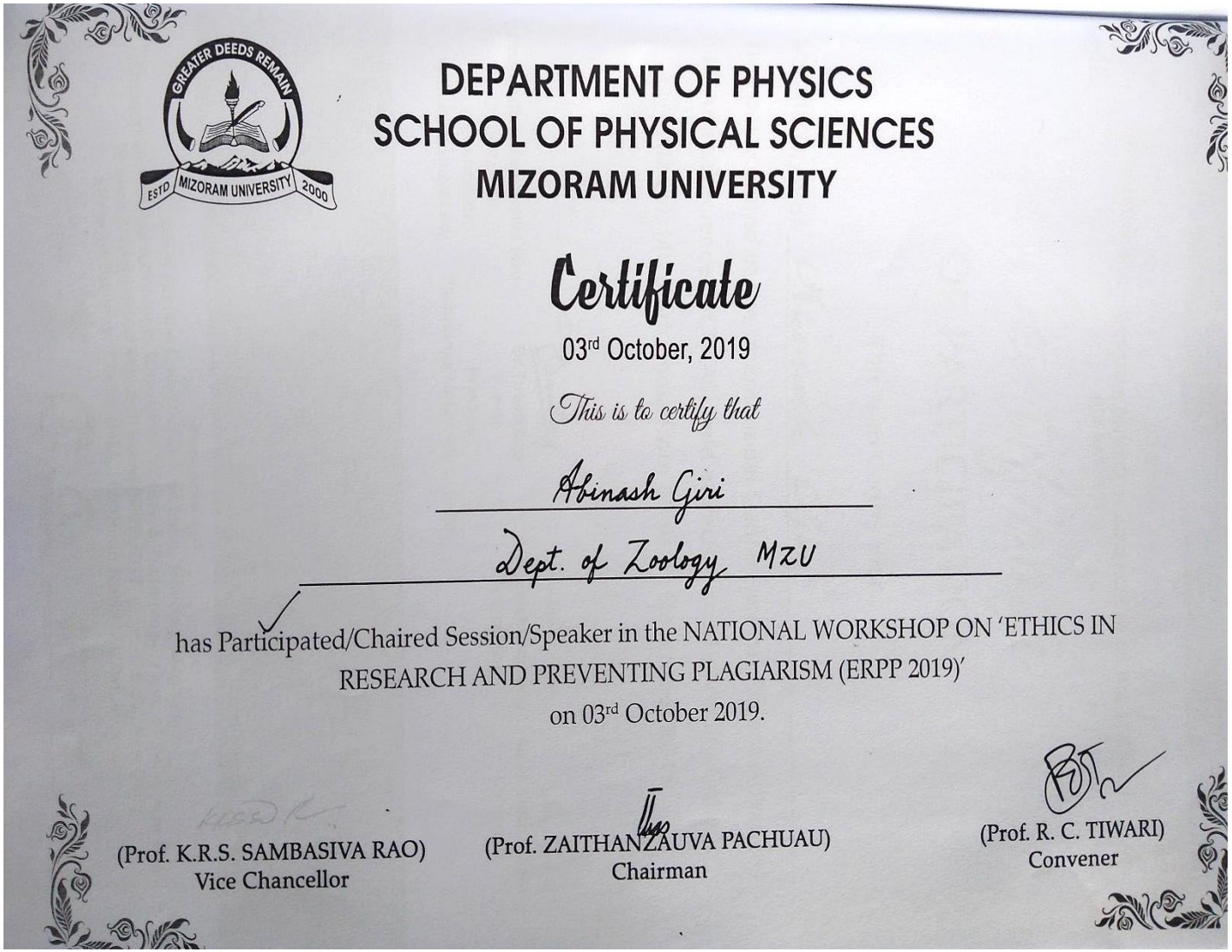
~~Resource person~~ in the National Workshop on Bioinformatics for

Zoologist *held during* 26 - 31 Aug 2019 *organized*

by Bioinformatics Infrastructure Facility (BIF), Department of Biotechnology, Mizoram University sponsored by Department of Biotechnology (DBT), New Delhi.

(Prof. K.R.S. Sambasiva Rao)
Vice-Chancellor
Mizoram University

(Prof. N. Senthil Kumar)
Coordinator
Bioinformatics Infrastructure Facility (BIF)
Mizoram University



DEPARTMENT OF PHYSICS
SCHOOL OF PHYSICAL SCIENCES
MIZORAM UNIVERSITY

Certificate

03rd October, 2019

This is to certify that

Abinash Giri

Dept. of Zoology MZU

has Participated/Chaired Session/Speaker in the NATIONAL WORKSHOP ON 'ETHICS IN RESEARCH AND PREVENTING PLAGIARISM (ERPP 2019)' on 03rd October 2019.

(Prof. K.R.S. SAMBASIVA RAO)
Vice Chancellor

(Prof. ZAITHANZAUVA PACHUAU)
Chairman

(Prof. R. C. TIWARI)
Convener

INTERNATIONAL CONFERENCE ON RECENT ADVANCES IN ANIMAL SCIENCES
(ICRAAS- 2019)

Certificate
OF PARTICIPATION

This is to Certify that

Ms/Mr/Dr/Prof Abinash Giri

has participated at the International Conference on Recent Advances in Animal Sciences (ICRAAS) held at Pachhunga University College, Aizawl, Mizoram, India from 6th to 8th November 2019.

Prof. K.R.S. Sambasiva Rao
Chief Patron
Vice Chancellor
Mizoram University

Dr Tawnenga
Patron
Principal
Pachhunga University College

Dr K. Lalchhandama
Chairperson

Dr H. Lalthanzara
Convener

Organised by:
Department of Zoology, Pachhunga University College, Aizawl, India

Co-Organisers :
Mizo Academy of Sciences (MAS)
Mizoram University, Tanhril, Aizawl
Directorate of Fisheries, Govt. of Mizoram
Directorate of Agriculture (Research & Education), Govt. of Mizoram
Environment, Forest & Climate Change Department, Govt. of Mizoram

Sponsored by:
North Eastern Council, Govt. of India, Shillong, Meghalaya
Department of Biotechnology, Ministry of Science & Technology, Govt. of India, New Delhi
Science & Engineering Research Board (SERB), DST, Govt. of India, New Delhi
Mizoram Business Centre (MBC), Ramhlun North, Aizawl, Mizoram

CERTIFICATE *OF* PARTICIPATION

UGC STRIDE PROGRAM
MIZORAM UNIVERSITY
Aizawl, India

THIS ACKNOWLEDGES THAT

ABINASH GIRI

attended Webinar presentation on

"Antibiotic Resistance"

On 17th July 2020 @ 11 AM

Speaker

Dr. Murthy S. Karnam
Professor, Department of Physiology and Biophysics
Virginia Commonwealth University
Richmond, VA 23298, USA

Prof. G. Gurusubramanian
Organizing Secretary
Department of Zoology

H. T. Lalremsangi
Organizing Secretary
Department of Zoology

Organizing Committee Members

Pratima Khandayataray, Meesala Krishna Murthy, & Abinash Giri, Dept. of Zoology, MZU

Mizoram University Webminar / Online Lecture Series

ABSTRACT

Population and genetic diversity in banana pseudostem weevil and leaf and fruit scarring beetle, inferred from RAPD fingerprints

An abstract submitted in partial fulfilment of the requirements for the Master of Philosophy

ABINASH GIRI

MZU Regn. No. - 1904355

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April 2021

ABSTRACT

Population and genetic diversity in banana pseudostem weevil and leaf and fruit scarring beetle, inferred from RAPD fingerprints

BY

ABINASH GIRI

Department of Zoology

Prof. GURUSWAMI GURUSUBRAMANIAN

Submitted

In partial fulfilment of the requirement of the Degree of Master of Philosophy in Zoology of Mizoram University, Aizawl.

INTRODUCTION

1.1 Banana cultivars

Banana is deliberated as one of the vital food items by means of it is high in nutrients and profitable value all over the tropical world. It is considered as a significant tropical natural product crop disseminated in 120 nations with a yearly production of 120 million tonne (FAO, 2005). Banana has a place with the family Musaceae, Zingiberales order, which comprises of two genera Musa L. and Ensete Bruce. It is profoundly expanded all through the world however answered to have begun from Southeast Asia (Simmonds, 1966). The genus Musa comprises of about 50 species whereas Ensete has 9 species (Azhar and Heslop-Harrison, 2008; Simmonds and Shepherd, 1955). Based on phenotypic qualities and essential chromosome number, Musa has been separated into four areas specifically, Eumusa, Rhodochlamys, Austra-limusa and Callimusa (Simmonds and Shepherd, 1955). The Eumusa comprises the wellsprings of eatable bananas got from the two wild diploid ancestor species M. acuminata (assigned as AA genome) and M. balbisiana (assigned as BB genome) which prompts the root of various ploidy levels of banana assortments (AB, AAB, ABB and ABBB) through intra and between explicit crosses(Uma et al., 2006). India is the biggest maker with the yearly creation of 13.5 mt from a territory of 400,000 lakh ha (Sathiamoorthy et al., 2001).

Northeast India has been considered as the richest sources of banana diversity in which the clones of M. balbisiana from Indian subcontinent hybridize with M. acuminata from Southeast Asia (Molina and Kudagamage, 2002). Information of the status of genetic resources of plants is a necessary for its proper understanding and implementation of maintenance and sustainable utilization. Wild and cultivated bananas are abundantly available in Mizoram, which is one of the states of Northeast Indian region (Lalrinfela and Thangjam, 2012).

Table 1. List of edible banana found in Mizoram

Scientific Name	**Edible banana**	**Genome Type**	**Morphological score (IPGRI, 1996)**
Musa paradisiaca	Banria	ABB	**62**
	Balhlasen	AAB	**38**
	Banthur	AAB	**39**
	Banpawl	AB	**59**
	Lawngbalhla	AAB	**33**
	Kawlbalhla	ABBB	**66**
	Vaibalhla	AABB	**60**
	Khumtungbalhla	AAB	**50**
	Zobalhla	AAB	**48**
	Changkha	AABB	**46**
	Kawrmuat	AAAB	**35**

(Uma *et al.*, 2001)

Table 2. List of wild banana cultivars found in Mizoram

Wild banana	Scientific Name	Genome Type	Morphological score (IPGRI, 1996)
Changthir	*Musa balbisiana*	BB	**70**
Changpui	*Musa acuminata*	AB	**46**
Changpawl	*Musa paradisiaca*	ABBB	**50**
Changvandawt	*Musa ornata*	--	**-**
Lairawk	*Musa paradisiaca*	AB	**46**
Lairoop	*Musa paradisiaca*	AB	**47**
Saisu	*Ensete glaucum*	--	--

(Uma *et al*., 2001)

1.2 Banana pests

Banana is attacked by number of pests, among them banana pseudostem weevil Odoiporus longicollis (Oliver) and leaf- and fruit- scarring beetle Basilepta subcostata (Jacoby) are major monophagous pests which limiting the production and productivity, posing serious threat to banana production (Visalakshi et al., 1989; Valmayor et al., 1994; Shukla and Kumar, 1970; Prathapan et al., 2019). O. longicollis causes damage that ranges from 10 % to nearly 90 % subject upon the phase of plant growth at which pest infestation occurs and the yield of the management practices that are being followed (Padmanaban and Sathiamoorthy, 2001). In addition to these, leaf- and fruit-scarring beetles (Coleoptera, Chrysomelidae) are key periodic pests of bananas and plantains in many states of northern, eastern, and north-eastern India, Bangladesh, and parts of Southeast Asia (Prathapan et al., 2019).

1.3 Population dynamics of Odoiporus longicollis and Basilepta subcostatum

The study of population and occurrence of O. longicollis is very much important for understanding the status and rate of population according the season and also to establish a well-developed control measures of this pest (Azam et al., 2010). O. longicollis is popularly known as an internal feeder pest of banana crop. All life stages of O. longicollis are remains active for throughout the year. There is no any hibernation period for them. The adult male and female weevils can reproduce in both summer and winter seasons (Devi et al., 2015). They present inside the pseudostem for all seasons but depending on the season the rate of population varies. The activity of O. longicollis became more during July to September i.e. in the monsoon period and gradually slows down from November to January (Priyadarshini et al., 2015). Based on the population rate of O. longicollis the rate of infestation can be studied (Thippaiah et al., 2010). The initial incidence of O. longicollis generally found from six month old plantation and with the growing stages of the banana crop the population of O. longicollis also increases gradually. The population rate of O. longicollis also very much dependent on the meteorological parameters such as maximum temperature, minimum temperature, relative humidity, rainfall (Biswas et al., 2015).

1.3 Life cycle of Odoiporus longicollis

The life cycle of the O. longicollis from egg to the adult were seen in banana pseudostem in summer and winter season (Padmanaban and Sathiamoorthy, 2001). O. longicollis infested cultivars can be recognized so easily by the help of small holes made by the weevils while feeding upon the pseudostem. These holes are found in the pseudostem of the cultivar. The elliptical, yellowish white eggs are laid by the female adult weevil under the outer leaf sheath of pseudostem. During the complete life cycle of the banana pseudostem weevil the larvae pass through 4th instar in the developmental stage. The apodous larvae are fleshy and yellowish white in colour. After the completion of 4th instar stage of the larvae they stops feeding and starts resting for pupa stage of their life inside self-made cocoon. Pupa develops into adult or the last life stage of the weevil. The adults are generally 20-30 mm long in size. In India both the black and red coloured adults are available (Priyadarshini et al., 2014). The colour differences in adult weevils are not because of the sexual dimorphism. According to the mating studies it is the result of the phenomenon of non-sex limited variation and of sympatry (Dutt and Maiti, 1972).

1.4 Life cycle of Basilepta subcostatum

The egg is oval in shape and the colour of it is pale lemon yellow. The larva is white with dark coloured head. During the complete life cycle of scarring beetles they passed their maximum life stages in the soil. They used to lay eggs in the soil and pupation also occurs in the soil, only the adults are found inside the leaf whorl. As they are known as night loving insects, during day time they hide themselves inside the curled leaf until any interference by anyone. The population of the scarring beetle are too high during the rainy seasons i.e. from April to September (Sharma and Saikia, 1967). Winter is the season of hibernation for the adult beetles.

1.5 Random Amplified Polymorphic DNA (RAPD)

For the study of genetic diversity in different organisms Random Amplified Polymorphic DNA (RAPD) fingerprinting have been used which is a PCR-based technique. The amplification products produced by RAPD primers anneal to homologous target sites of the template DNA, in which the genomes are randomly distributed (Williams et al., 1990; Welsh and McClelland, 1990). RAPDs are delicate enough to notice dissimilarities between individuals showing a close genetic relationship. The key benefit of this method is that it can be useful with few necessities for modelling, assumptions or analysis (Black IV, 1993). Besides, this technique has been confirmed to be useful in exposing geographical origins and scattering routes of insect pest populations, mostly curculionid weevils (Taberner et al., 1997; Bas et al., 2000; Scataglini et al., 2000; Kim and Sappington, 2004).

To study the population of numerous insects, mitochondrial genes have been used because they have numerous significant characteristics i.e. those are haploid, inherited from maternal, absence of introns, progress more quickly than nuclear coding genes, deficiency of recombination and are expected to differ in a neutral manner. Comparing 12S and 16S rDNA genes the mitochondrial protein coding region rate of evolution is much faster and hence those regions help as useful markers for reading evolutionary history at the periods of

family, genera and species (Wan et al., 2004). Interpreting phylogeny and phylogeography mitochondrial genes have been used in several insects (Orsini et al., 2007) including Heliconius butterflies (Brown, 1994), Halys fabriciusm (Memon et al., 2006), Diabrotica (Szalanski et al., 2000), Adelges cooleyi (Ahern et al., 2009), Aphidus ervi (Hufbauer et al., 2004) and Apis cerana indica F (Baskaran, 2011).

In limited studies, mitochondrial genes have not shown useful for the assessment of phylogeography at the intraspecies level. For example, intraspecific phylogeography of Apis cerana did not associate with geographic distribution when COI/COII area was used for association of dissimilar geographic populations (Hepburn et al., 2001). In Tomincus destruens Woll Horn et al. (2006) gained no clear phylogeographic pattern within geographic populations.

1.6 Mitochondrial Cytochrome Oxidase I Marker

Compared to nuclear markers, mitochondrial markers are more susceptible to the effects of genetic drift (Filipova et al., 2011). As a powerful and widely used molecular marker, mtDNA has been applied in many organisms to determine the genetic variations and structure of population (Xu et al., 2011). Mitochondrial DNA has become a major tool of comparative genomics and occupies a significant role in genetic structure of population and molecular variations as it is maternally inherited with no intermolecular genetic recombination with rapid rate of evolution (Near et al., 2003; Cardenas et al., 2009; Xu et al., 2011). COI is a protein-coding gene in mtDNA. Due to fast evolution, high polymorphism, easy amplification and sequencing, it has shown valuable information and is a widely used genetic marker for population genetic studies especially intra-specific analysis (Near et al., 2003; Hu et al., 2008; Cardenas et al., 2009; Xu et al., 2011).

Mitochondrial markers are more sensitive Compared to nuclear markers for the effects of genetic drift (Filipova et al., 2011). mtDNA has been worked as an influential and extensive molecular marker in many organisms to govern the genetic variations and structure of population (Xu et al., 2011). Mitochondrial DNA has converted to a major tool of relative genomics and occupies an important role in genetic structure of population and molecular dissimilarities as it is inherited from maternally with no intermolecular genetic recombination with rapid rate of evolution (Near et al., 2003; Cardenas et al., 2009; Xu et al., 2011). COI is a broadly used genetic marker because it is a protein-coding gene of mitochondria having rapid evolution, high polymorphism and easy amplification and sequencing (Near et al., 2003; Hu et al., 2008; Cardenas et al., 2009; Xu et al., 2011).

REVIEW OF LITERATURE

2.1 Banana cultivars of Mizoram

The economic review for the identification of the potential banana cultivars inborn to Mizoram was identified as Vaibalhla (AAA), Banria (ABB) and Lawngbalhla (AAB). The most popular cultivar with the maximum economic value was Vaibalhla. The cultivar of Vaibalhla is a triploid *M. acuminata* (AAA) of the Cavendish sub-group having a sweet taste (Lalrinfela and Thangjam, 2012). It has been described by Lescot (2011) that half of the current banana production depend on somaclones derived Cavendish dessert bananas (AAA group). The most vital qualities that make the Cavendish sub-group the main bananas for transfer are related to their consistency during transport and their shelf life. Between the 3 cultivars identified, 2 (Banria and Lawngbalhla) were found throughout the state while Vaibalhla was found only in the tropics (Hrahsel and Thangjam 2013). It is a clear known point that banana (*M. acuminata*) having the A genome are more cold-sensitive as associated to plantains (*M. balbisiana*) having the B genome, even though the biological mechanisms of cold-tolerance for plantains are still not clear (Zhang *et. al*., 2011). The Vaibalhla (AAA) was not found in the temperate region of Mizoram (Champhai district), while Banria (ABB) and Lawngbalhla (AAB) flourishes well in all the phytogeographical regions including the temperate regions (Hrahsel and Thangjam 2013).

2.2 Biology and population of *Odoiporus longicollis*

An widespread record on the biology and pest status of the banana pseudostem weevil, *O. longicollis*, was recorded by Dutt and Maiti (1972), in this way Isahaque (1978), Shukla and Tripathi (1978), Visalakshi *et al.* (1989), Padmanabhan and sathiamoorthy (2001), Tiwari *et al*. (2006) and Thippaiah *et al*. (2010), furthermore exposed the biology of *O. longicollis* all in all. Azam et al. (2010) by their examination to discover the event, mode, degree of harm, life design of pseudostem weevil of banana filled in Poonch and Rajouri regions of Jammu and the vermin population during several seasons and field conditions it has been suggested that to

strategy for a practical control measure, information on the frequency of the impatience and the number of populaces in the weevil during various seasons is a lot of important. Priyadarsini et al. (2014) considered about bioecology and seasonal incidence pattern *O. longicollis* in cooperation with field and laboratory conditions. Krishnan and Jayaprakas (2015) intense on the bionomics, circulation and the executives of the banana pseudostem weevil and determine that impulsive utilization of compound assistances to farming poses dare to practical agriculture is possible simply by considerate the pests in its morphological, taxonomical, natural and distribution levels. The way of occurrence of *O. longicollis* was studied by field inquiry under gangetic tract of West-Bangle (Biswas et al., 2015). Devi et al. (2015) stated that *O. longicollis* are found active in all seasons and maximum in the month of September through their study on the population structure and seasonal incidence of the pest.

The leaf sheaths have spaces where mating occurs and in the one air chamber present inside the leaf sheath they laid one egg. The shape of egg is cylindrical and yellowish white in colour. Larvae consist of five larval instars and they are apodous, soft having dark brown head. Extended cylindrical cocoons are made for pupation by twisting short pieces of chewy materials of the leaf sheath. The adults were Black and reddish brown noted from disassociated pseudostem of infested banana cultivars. The weevil breeds all over the year and do not go through winter rest (Azam *et al*., 2010).

Incongruencies detected in the phylogenies constructed on mitochondrial and nuclear genes have completed studies found on both groups of genes significant in insect molecular systematics (Shankar *et al*., 2015). Supervision of *O. longicollis* is a criterion to satisfying productivity and to obtaining higher economic profits of bananas and plantains. A serious aspect for evolving a successful integrated pest management (IPM) policy for the regulator of this pest is the study of the population structure of the pest, i.e. measuring the genomic variability of the pest among and within sites and how this variability is separated geographically. The internal transcribed spacers of I and II of rDNA have been used to measure the genetic

diversity of *O. longicollis* entities composed from six hotspot locations in India (Kumar *et al*., 2018).

The larvae and pupae those live in inside the pseudostem can endure the storage circumstances during transport. Biological structures increase the chance of establishment of these weevils in fresher areas (Kumar *et al*., 2018). 2-Methyl-4-heptanol (2M4H) was testified as the male-secreted combination pheromone of *O. longicollis* (Gunawardena *et al*., 1997). Male weevils were reactive to male as well as female extracts whereas female receptive towards only male extract (Prasuna *et al*., 2008). The pests breed throughout the year and do not undergo winter rest (Azam *et al*., 2010).

Williams *et al*., (1991) defined a technique that they called RAPD (Random Amplified Polymorphic DNA) in which a ten oligonucleotide primers of random sequence but with a least of 50% guanine-cytosine contents. The polymerase chain reaction (PCR) is a highly effective technique of amplifying distinct DNA fragments using a thermo stable DNA polymerase with single-stranded DNA primers. An application of the PCR technique that uses DNA primers of arbitrary nucleotide sequence to amplify arbitrary regions of the genome has been described (Welsh *et al*., 1991 and Williams *et al*., 1991).

2.3 Biology and population dynamics *Basilepta subcostatum*

An investigate was considered in the natural plantation of banana to scrutinise the population structure of leaf and fruit scarring beetles, *Basilepta subcostatum* found in Assam Agricultural University, Jorhat (Mishra *et al*., 2015). To know the periodical occurrence of *B. subcostatum* on banana plantation Sah *et al*. (2018) considered on the population build up and invasion of *B. subcostatum*. By this experiment they found that the population construction of this pest in dependent on temperature and at low temperature the population rate goes down and as well as infestation rate also. To determine the structural composition of different species of leaf and fruit scarring beetle in the northern and northeastern regions of India, Prathapan et al. (2019) studied on species configuration of this pest through taxonomy and COI sequence analysis.

2.2 RAPD

Genetic distance matrices generated by RAPDs are low level of connection RAPDs and revealed polymorphisms in the coding as well as in the non-coding regions and can possibly cover the whole genome (Shankar *et al*., 2014). The UPGMA dendrogram resulting from RAPDs clusters in the individuals rendering to the sampling locations and AMOVA analysis displays that nearly half of the observed genetic variation happens within the populations and this indicate that the banana rhizome weevil had formed local populations due to limited dispersal (Yadav *et al*., 2017).

2.3 Mitochondrial COI marker

The gene mutation inherited from maternal mitochondrial genome was frequently derived from different sequence. The study of intraspecific polymorphism of COI is valuable information derived from mitochondrial genome (Barbaresi *et al*., 2003). The COI sequence provides well Understanding of characteristics of genetic structure population (Liu *et al*., 2013).

The L2 gene of insects and crustaceans, encoding the two codon families of UUR, lies among the COI and COII genes. The COI-tRNA^{Leu} -COII sequenced region displayed the distinctive AT bias as observed in insect mtDNA (Frati et al. 1997). The GA and CT (U) transitions were extra frequent than the T(U)C and AG transitions (Shankar *et al*., 2014).

Though *O. longicollis* is a serious pest of bananas, there are no sufficient reports on the characterisation of this pest using molecular markers Northeast India.

OBJECTIVES

- Biology, seasonal abundance, population dynamics and host preference of banana pseudostem weevil, *Odoiporus longicollis* and banana scarring beetle, *Basilepta subcostatum* in banana growing regions of Mizoram.

- Host- based genetic differentiation of *O. longicollis* by using RAPD markers.

- Verification of mitochondrial COI markers to recognize phylogeographical relationships among *O. longicollis* and *Basilepta subcostatum* to reveal their population genetic structure.

MATERIALS AND METHODS

4.1 Survey and collection of the samples

The circulation of banana varieties in different parts of Mizoram was got from the discussion of the concerned officials of agriculture and horticulture departments of Mizoram, and the local farmers. *O. longicollis* and *B. subcostatum* were collected from the banana cultivars from the four districts (Aizawl, Lunglei, Lawngtlai and Saiha) (**Table 4**). Specimens from different populations were collected by directly under the leaf sheath of recently infested trees. Collected beetles were kept in 70% ethanol in -20 ºC (Yadav *et al*., 2017).

4.2 Identification and characterization of Banana cultivars in Mizoram

The taxonomical classification and identification of the collected banana cultivar samples were carried out by assessing the habit, leaf, floral and fruit features using the identification keys provided by Singh *et al*. 2012 and Häkkinen, 2013. For genome classification, the morphological characters of vegetative, male and female inflorescence based on 15 characters suggested by Simmonds and Shepherd 1955 were evaluated (**Table 3**) and a relative score was recorded (Uma *et al*., 2001; IPGRI, 1996). For example, with respect to pseudostem colour, score of 1 is given, if the pseudostem is heavily blotch with brown or black pigmentation. Similarly, a maximum score of 5 was given when blotches are completely absent and the pseudostem is more or less green. Intermediary scores from 1-5 depending on the extent of blotching and the score range from 1-75 (**Table 3**).

Table 3. Morphological characters used for banana classification (Simmonds and Shepherd, 1955).

Sl No	Characters	*Musa acuminate*	*Musa balbisiana*
1	Pseudostem colour	More or less heavily marked with brown or black blotches	Blotches slight or absent
2	Petiolar canal	Margin erect or spreading, with scarious wings below, not clasping pseudostem	Margin enclosed, not winged below, clasping pseudostem
3	Peduncle	Usually downy or hairy	Glabrous
4	Pedicel	Short	Long
5	Ovules	Two regular rows in each loculus	Four irregular rows in each loculus
6	Bract shoulder	Usually high (ratio < 0.28)	Usually low (ratio < 0.30)
7	Bract curling	Bract reflex and roll back after opening	Bract lift but do not roll
8	Bract shape	Lanceolate or narrowly ovate, tapering sharply from the shoulder	Broadly ovate, not tapering sharply
9	Bract apex	Acute	Obtuse
10	Bract colour	Red, dull purple or yellow outside; pink, dull purple or yellow inside	Distinctive brownish-purple outside; bright crimson inside
11	Colour fading	Fading inside bract colour fades to yellow towards the base	Inside bract colour continuous to base
12	Bract scars	Prominent	Scarcely prominent
13	Free tepal of male flower	Variably corrugated below tip	Rarely corrugated
14	Male flower colour	Creamy white	Variably flushed with pink
15	Stigma colour	Orange or rich yellow	Cream, pale yellow pale pink

Table 4. List of geographical population

Sl No	Location	Coordinates	Districts	Banana Variety	*Musa* sp.
1	Tanhril 1	23.737, 92.663	Aizawl	Changthir	*balbisiana*
2	Tanhril 2	23.734, 92.668		Balhlasen	*paradisiaca*
3	Tanhril 3	23.737, 92.670		Banria	*paradisiaca*
4	Tanhril 4	23.737, 92.701		Vaibalhla	*paradisiaca*
5	Tuirial	23.759,92.635		Changpawl	*paradisiaca*
6	Sakawrtuchhum	23.759, 92.651		Changpui	*acuminata*
7	Tanhril 5	23.737, 92.663		Vaibalhla	*paradisiaca*
8	Tanhril 6	23.737, 92.663		Banria	*paradisiaca*
9	Tanhril 7	23.737, 92.663		Banria	*paradisiaca*

10	Theriat	22.735, 92.471	Lunglei	Zoblhla	*paradisiaca*
11	Theriat	22.731, 92.465		Khumtungbalhla	*paradisiaca*
12	Theriat	22.731, 92.465		Khumtungbalhla	*paradisiaca*
13	Tuipui	22.879, 92.935		Khumtungbalhla	*paradisiaca*
14	Darzo	22.833, 92.955		Khumtungbalhla	*paradisiaca*
15	Vanlaiphal	22.803, 92.995		Vaibalhla	*paradisiaca*
16	Sangau	22. 441, 93.410	Lawngtlai	Khumtungbalhla	*paradisiaca*
17	Cheural	22.707, 93.015		Vaibalhla	*paradisiaca*
18	Cheural	22.707, 93.015		Khumtungbalhla	*paradisiaca*
19	Rawlbuk	22.673, 92.996		Vaibalhla	*paradisiaca*
20	Saiha	22.489, 92.979	Saiha	Vaibalhla	*paradisiaca*

4.3 Life cycle

Life cycle of the insect was studied in the laboratory conditions (27 ± 3 °C, 60 ± 10% RH and L:D 12:12). Mating behaviour, pre-oviposition and oviposition behaviour, egg and incubation period, larva, feeding behaviour, pupation, pupa and adults were studied. Images were taken by camera under necessary zoom. Fecundity Cocoons collected from the infested plants were individually reared in 100ml plastic cups with in emergence, one male and one female each of 13 days old was confined for mating in a 100 mL plastic container for 24 hours, and was provided pseudo stem pieces of 4 x 3 cm for feeding and egg laying. In order to understand the mating frequency and fecundity, two sets of experiment was conducted; one with female exposed to male only for 24 h whereas in the other set male and females will exposed continuously till their death. Five replications were maintained (Krishnan and Jayaprakas, 2015).

4.4 Population and infestation studies of *Odoiporus longicollis* and *Basilepta subcostatum*

The size of population and infestations studies of *Odoiporus longicollis* and *Basilepta subcostatum* were conducted from August, 2019 to January, 2021 in four district of Mizoram. Arbitrarily four banana orchards were selected from each site. The population of *O. longicollis* and *B. subcostatum* were studied from haphazardly selected plants. The *O. longicollis* population was studied by taking account of

weevils on pseudostem and holes created by the weevils (i.e. per 30cm^2 area) from the number of holes the pseudostem was studied. For *B. subcostatum* the overall size of population was calculated the number of beetles found on leaf surface and inside the cigar. Infestation pattern was counted by the sum up the number of scars presented on per 5cm^2 area of banana leaf surface (Mishra *et al.*, 2015).

4.5 RAPD fingerprints of *O. longicollis*

4.5.1 DNA extraction

DNA was extracted by using Sambrook and Russell (2006) with some modifications. Samples were washed twice with phosphate buffered saline (PBS) (500 µL), centrifuged at 12,000 rpm for 10 min and dried. 300 µL of buffer and TEX buffer was added respectively, to the sample placed in mortar pestle and crushed the samples. After crushing 10 µL proteinaseK was added in each tube and vortex was done vigorously in each tube for 1 min and kept for overnight incubation at 55 °C in thermo cycler at 1300 rpm. The samples were kept in room temperature for 10 min centrifuged at 14,000 rpm for 10 min. Took supernatant in separate tube and added 500 µL of phenol: chloroform: isoamyl alcohol (25:24:1). The solutions were mixed by inverting for 2 min and centrifuged at 14,000 rpm for 10 min. Supernatant was taken in the fresh tubes and discard pellet. 400 µL chilled isopropanol was added and slowly until white flakes appear and incubated at -20 °C for 1 h. After incubation kept samples in room temperature for 5 min and centrifuged at 10,000 rpm for 10 min. The supernatant was decanted and 400µL of 95% chilled ethanol and sodium acetate 100 µL was added to the pellet for washing and tapped for 5 min. Supernatant was decanted and dry the pellet in room temperature. Added 20 µL of nuclease free water in tube and stored in -20 °C for further use.

4.5.2 RAPD-PCR amplification

A total of 19 decanucleotide RAPD primers of the series A 3 primers(OPA-05, OPA-12, OPA-20), B 3 primers(OPB-05, OPB-08, OPB-19), E 5 primers(OPE-04, OPE-06, OPE-08, OPE-11, OPE-13) and G 8 primers(OPG-06, OPG-08, OPG-09, OPG-11, OPG-12, OPG-14, OPG-15, OPG-16) obtained from Operon Technologies Inc. (Alameda, CA, USA) primarily screen for identifying primers that was give

clear amplification products. RAPD-PCR standardized with respect to the concentration of DNA, RAPD primer, temperature of annealing, TaqDNA Polymerase (Yadav *et al.,* 2017). The PCR reactions carried out in a total volume of 25 µL containing 3 µL of genomic DNA, 3.2 µL (1.25 mM) dNTPs, primer 0.4 µL, PCR buffer 2 µL, 1.5 U of Taq DNA Polymerase (Bangalore Genei, India) of 0.2 µL, and BSA 2 µL, 2.8 µL $MgCl_2$ (25 mM) and 6.4 µL nuclease free water. The PCR cycle conditions for RAPD-PCR included an initial denaturation at 92-95 °C for 5 min followed by 35-39 cycles each of a denaturation step at 94 °C for 4-5 min, 94 °C for 30 s- 1 min; annealing at 32-65 °C for 30 s- 1 min; extension at 72 °C for 1-2 min followed by a final extension at 72 °C for 5-10 min. The PCR products were run in a 1.5 - 2% agarose gel stained with the help of ethidium bromide 3 µL (0.5 µg/mL) (Yadav *et al.,* 2017).

4.6 Amplification of the Mitochondrial DNA COI

The mtDNA COI fragment was amplified from separate weevils using the primer pair COI-LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and COI-HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). The PCR reactions carried out in a total volume of 25 µL containing 2 µL of genomic DNA, 3.2 µL (1.25 mM) dNTPs, LCO1490 primer 0.4 µL, HCO2198 primer 0.4 µL, PCR buffer 2 µL, 1.5 U Taq DNA Polymerase (Bangalore Genei, India) of 0.2 µL, and BSA 2 µL, 2.8 µL $MgCl_2$ (25 mM) and 7 µL nuclease free water. The PCR cycle conditions for RAPD-PCR included an initial denaturation at 94 °C for 5 min followed by 35 cycles each of a denaturation step at 94 °C for 2 min, 94 °C for 30 s; annealing at 51 °C for 30 s; extension at 72 °C for 2 min followed by a final extension at 72 °C for 5 min. The PCR products were run in a 1.5% agarose gel stained with the help of ethidium bromide 3 µL (0.5 µg/mL) (Hebert *et al*., 2004).

Successful amplified DNA templates were sent for Sanger sequencing on ABI 3730XL sequencer at AgriGenome sequencing facilities (Kochi, Kerala). For Sanger sequencing both directions and fragments were assembled to form contigs by Geneious V11.0.4 (Kearse *et al*., 2012), then aligned and visually checked for quality and noise to resolve some of the ambiguities. For each sample, we ensured there was no pseudogenes presence similarly to HTS sequences, and we checked for

possible cross-contamination by blasting sequences on BOLD to test similarity with conspecific and congeneric existing records. Low quality of electropherograms (potentially due to low DNA concentration, DNA degradation or contaminantion) was discarded. The sequences were deposited in GenBank with the following accession numbers for COI: KJ446900.1 to KJ446919.1.

4.7 Sequence analysis by MEGA

DNA sequence chromatograms were read and discrepancies between forward and reverse sequences were resolved using the Chromas software v 2.01 (http://www.technelysium.com.au/chromas.html). MUSCLE was used to generate the alignments (Edgar, 2004). The sequences were imported into MEGA X for analysis of model test: 0-fold, 2-fold and 4-fold degenerate sites, Estimate of the pattern of nucleotide substitution in COI sequences, estimates of base composition bias, Neutrality analysis: Tajima's test statistics and phylogenetic analysis (Tamura *et al*., 2007). Statistical support for the inferred nodes was obtained by bootstrapping in MEGA X (Tamura *et al*., 2007).

RESULTS

- Nine different banana cultivars (Banria, Balhlasen, Changthir, Changpawl, Changpui, Khumtungbalhla, Lawngbalhla, Zobalhla and Vaibalhla) were identified and characterized by using standard protocols and performed the bioecological studies of *O. longicollis* and *B. subcostatum* in twenty sites of four districts of Mizoram.
- The time period observed from egg to the adult banana pseudostem weevil was found to be 46 ± 7.2 (35 to 58) days in summer (May to August) and 64 ± 11.4 (47 to 85) days in winter (November to February).
- Population dynamics study of *O. longicollis* discovered that in rainy season with high temperature reduced the infestation rate along with decreased population size. The population size and infestation pattern rate of the *O. longicollis* were negatively correlated with meteorological parameters.
- The infestation pattern rate and population distribution of *B. subcostatum* increased during summer season. The population and infestation rate was maximum in the month of August and lowest in December. The population size and infestation of the *B. subcostatum* were positively correlated with meteorological parameters.
- Infestation pattern and population distribution (larva, pupa and adult) of *O. longicollis* and *B. subcostatum* were detected to be high in *Musa balbisiana* (Changthir) followed by *Musa acuminata* (Changpui), *Musa paradisiaca* (Changpawl), in the chosen locations of four districts of Mizoram.
- The randomly amplified polymorphic DNA (RAPD) marker study displayed no amplification results with 19 primers both in *O. longicollis* and *B. subcostatum*.
- Because of no results found in RAPD analysis as an alternative COI marker was chosen for population genetic structure analysis of *O. longicollis* and *B. subcostatum*.

- Based on low BIC, AIC and InL scores the Tamura 3 parameter model was assessed as the finest model for COI gene sequence of both *O. longicollis* and *B. subcostatum*. Gamma parameter value i.e. site rate variation, was found to be high in A and T than C and G.
- In *O. longicollis*, it was found that 817 conserved site, 18 variable sites, and 8 parsimony informative sites, 10 singleton sites out of 835 nucleotides of the CO1 gene and in case of *B. subcostatum*, 396 conserved sites, variables sites 159, 1 parsimony informative sites, 158 singleton sites out of 674 nucleotides.
- Estimation of base composition bias difference between twenty sequences in *O. longicollis* and six in *B. subcostatum* showed violation of the assumption of equality of substitution rates between geographical population and banana cultivars and substitution patterns are not homogenous ($P< 0.05$).
- The subsequent nucleotide composition analysis in COI was estimated that the frequency of AT was higher than the GC in all the twenty geographical population of *O. longicollis* and six geographical population of *B. subcostatum*.
- RSCU statistical analysis revealed that the codon usage bias was observed in nineteen codons out of 278 in COI gene of *O. longicollis* and seventeen codons out of 176 in COI gene of *B. subcostatum*.
- The average COI haplotype diversity ($h = 0.95$) in *O. longicollis* and ($h = 0.91$) in *B. subcostatum* specifies the effectiveness of COI sequence variation in detecting genetic structure.
- The nucleotide and amino acid variability of COI confirmed that the ratio of nonsynonymous to synonymous substitutions is high, indicating that COI gene is subject to strong positive selection.
- Estimation of synonymous and nonsynonymous substitution rates under realistic evolutionary models nonsynonymous is high in COI gene of *B. subcostatum* indicates a positive selection.
- Negative Tajima's (D) indicates an excess of low frequency polymorphisms relative to expectation, indicating population size

expansion and/or purifying selection in both *O. longicollis* and *B. subcostatum*.

- COI mitochondrial molecular marker identified four main haplogroups in twenty geographical populations of *O. longicollis* and six geographical populations of *B. subcostatum* respectively.
- Based on the population genetic structure analysis (Model test, transition/transversion bias, codon usage, substitution matrix, Tajima test of neutrality and phylogenetic trees) of COI sequence of *O. longicollis* and *B. subcostatum* revealed the fact that the effect of geographic isolation on genetic structure is the model of isolation by distance (IBD), which forecasts that genetic differentiation between twenty geographical populations and six geographical populations increases with geographic distances (Aizawl, Lunglei, Saiha and Lawngtlei).
- Further it is proved that there is a strong correlation between genetic variation in banana cultivars and host associated differentiation in COI genetic makeup of *O. longicollis* and *B. subcostatum*.