DIVERSE EFFECTS OF DEGREE OF URBANIZATION AND FOREST SIZE ON BEETLES BIODIVERSITY AND SPECIES COMPOSITION IN AIZAWL, MIZORAM

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Diverse effects of degree of urbanization and forest size on beetles biodiversity and species composition in Aizawl, Mizoram

BY

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Submitted in partial fulfillment of the requirement of the Degree of Master of Philosophy in Zoology, Mizoram University, Aizawl

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……., 2020

DECLARATION

I, **Malsawmdawngzuali Tara**, hereby declare that the subject matter of this dissertation entitled "**Diverse effects of degree of urbanization and forest size on beetles diversity and species composition in Aizawl, Mizoram**" is the record of work done by me, that the contents of this thesis 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 thesis has not been submitted by me for any research degree in any other university/Institute.

This is being submitted to the Mizoram University for the degree of Master of Philosophy in Zoology.

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This is to certify that **Ms. Malsawmdawngzuali Tara**, a M.Phil. Scholar, Registration No. **. MZU/M.Phil/531 of 17.05.2019** has worked on the dissertation entitled "**Diverse effects of degree of urbanization and forest size on beetles diversity and species composition in Aizawl, Mizoram".** She has fulfilled all the criteria prescribed by the UGC (Minimum Standard and Procedure governing Ph.D Regulation). She has fulfilled all the mandatories are required for M.Phil degree (enclosed). It is also certified that the scholar has been admitted in the department through an entrance test followed by an interview as per M.Phil - UGC Regulation, 2016.

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(MALSAWMDAWNGZUALI TARA)

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INTRODUCTION

Beetles belong to the order Coleoptera, the largest order of insects and has an estimated 3,50,000 species (www.zin.ru/Animalia/Coleoptera) that are classified into 160 families (Elzinga, 1992). These insects, which are characterized by their thick and tough forewings, or elytra, have adapted to various habitats from aquatic to soil, to trees, foliage, and other aerial surfaces. They can range in size from 1 mm to approximately 75 mm in length (Borror *et al*., 1976). In India alone, more than 15,500 species of beetles have been recorded (Sengupta and Pal, 1998).

Beetles play a vital role in the forest ecosystem due to their extreme sensitivity to ecological disturbances (Filgueiras *et al.,* 2011). Some of the beetle families which are found to be predominant in tropical forests have been employed as bioindicator species in biodiversity monitoring programs to assess forest cutting, alteration in landscape, habitat degradation, fragmentation-related effects, organization patterns change in species composition, farming intensification, and anthropogenic environmental disturbances (Audino *et al.,* 2014; Barnes *et al.,* 2014; Campos and Hernández 2015; Filgueiras *et al.,* 2011, 2015; Korasaki *et al*., 2013).

Beetles have also been known to be used in forest ecosystems where their species diversity and/or abundances change along a habitat disturbance gradient; and is found that while specialist species may decrease with increase in disturbance, the generalist species with good dispersal ability are increasing. At the same time, some species may not be affected by restrained disturbance.

In recent years, there has been a number of works which have been carried out on many Coleopteran families like, Staphylinidae, Scarabaeidae,Carabidae, Cicindelidae and Psephenidae to designate and use them as ecological indicators for

many purposes apart from other orders of class Insecta like Trichoptera , Ephemeroptera and Plecoptera. The presence and condition of beetles also give a more accurate information about the health of the ecosystem as their behaviour is directly related to the anthropogenic-induced landscape modification in the form of agricultural fields, plantations, and urbanisation. (Bhargava, 2009)

Urbanization refers to the population shift from rural areas to urban areas. It is the gradual increase in the proportion of people living in urban areas, and the ways in which each society adapts to this change. Urbanization is increasing globally and its related factors which include increase in spatial isolation and decrease in the habitat size can change the dynamics of plant and animal populations in urban green areas (Niemela, 1999; McKinney, 2002). There have been several studies along urbanization gradients which reported alterations in abiotic conditions in the remaining habitat patches caused by changes in temperature, precipitation and nitrogen deposition from the rural surroundings to the city centre (Bai *et al.,* 2008; Gilbert, 1994; Irwin *et al.,* 2011). All these changes can case a change in the habitat quality and, ultimately, to the species richness, composition and functional diversity of plants and animals (McKinney, 2002; Sukopp, 1998; ConcepcioÂn, 2015), which in turn affect the functioning of ecosystems (Sala *et al.,* 1997).

Anthropogenic activities that causes changes to the forest and landscape which pertains to towns and cities causes major changes to the ecosystem. Differences in a number of variables such as temperature (Bornstein, 1968), acidity, soil hydrophobicity, utilizable carbon and nitrogen levels (McDonnell *et al.*, 1997), deposition of heavy metals (Hynninen, 1986) and other pollutants (Herrmann and

Hübner, 1984; Väisänen, 1986), changes in fungal biomass, bacterial flora, leaf-litter decomposition rates (McDonnell *et al*., 1997), fragmentation and edge effect (Löfström *et al*., 1999), all contribute to the effect of urbanization. In addition, the highly significant physical covering of potential habitat with asphalt and cement causes direct loss of habitat.

The identification of the main factors that drive the composition of communities and the distribution of species is an essential objective in community ecology and is of particular importance for envisaging biodiversity responses to environmental changes (Belyea and Lancaster, 1999). Ozinga *et al*. (2009) showed that differences between plant species in characteristics involved in dispersal processes contribute significantly to explaining losses in plant diversity in response to habitat degradation.

Due to their extreme species richness, beetles, coupled with the morphological, as well as ecological and behavioural diversity, and are being widely used for environmental impact assessments, ecological studies and monitoring activities (Bicknell *et al*., 2014). While their significance for environmental impact assessment may be undisputed, there still remains a need to better understand the habitat requirements, especially of their larvae, as well as interactions between species (Rainio and Niemela, 2003). This often requires superior taxonomic tools. This is where DNA barcoding come into play as it can provide an efficient method for biodiversity assessment as it meets the need for a faster, more efficient and reliable species identification at this time of climate change and massive habitat destruction (Valentini *et al*., 2009). This approach may possibly be a much better

way for handling the vast diversity of invertebrates which are critical for ecosystem functioning while at the same time often poorly known taxonomically. DNA barcoding also has the power to connect different life stages of the insects. As such, it can link hundreds of years of taxonomic, ecological, faunistic and ethological studies with ultra-high-throughput sequencing of the genomic age. The latter approach will have great benefits for many application-oriented fields, especially in agriculture and forestry where the swift and reliable identification of bulk samples is often required.

REVIEW OF LITERATURE

An increase or a decrease of beetle species number or abundance might be directly caused by change in environmental factors or indirectly by change of species assemblage of other species (Rainio and Niemela, 2003).

Several urban studies reported a replacement by generalist species of forest specialists with an increase in the degree of urbanization which suggests that forest specialists are more sensitive to urbanization- related disturbances (Deichsel, 2006; Vergnes, 2014; Magura, 2010).

Some of the beetle families which are found to be prevalent in tropical forests include Scarabaeidae, Carabidae, Bruchidae, Buprestidae, Cantharidae, Cerambycidae, Chrysomelidae, Coccinellidae, Curculionidae, Elateridae, Lampyridae, Staphylinidae, Melolonthidae, Lucaenidae, and Tenebrionidae. They have been employed as bioindicator species in biodiversity monitoring programs to assess forest cutting, alteration in landscape, habitat degradation, fragmentation-related effects, organization patterns change in species composition, farming intensification, and anthropogenic environmental disturbances (Audino *et al.*,, 2014; Barnes *et al.*, 2014; Campos and Hernández 2015; Filgueiras *et al.*, 2011, 2015; Korasaki *et al.*, 2013).

The first report on the family cerambycidae from the state of Chhattisgarh accounts for 10 species of Cerambycid beetles belonging to eight genera and six tribes under two subfamilies (Majumder, 2014).

A study reports a number of new locality records for beetle species, which includes some from the state of Mizoram being recorded for the first time, of 92

species of *Sericini* (Coleoptera: Scarabaeidae: Melolonthinae) from the Indian subcontinent. There are a total of eight new species which are described which includes *Maladera alloservitrita* , *M. kolasibensis, M. mizoramensis, Neoserica radhanagariensis, Serica basantapurensis, S. mahakaliensis, S. therathumensis,* and *S. zianii* . (Shreedevi *et.al.,* 2018).

The number of cerambycid species recorded from India is about 1500 (Beeson 1939; Breuning, 1966) including 13 species reported from Tripura (Mukhopadhyay and Biswas, 2002). The pioneering taxonomic and biological investigations on cerambycid beetles in India were initiated in the 20th century. Gahan (1906) was the first to compile and describe the known cerambycid beetles, excluding Lamiinae, from the Indian region in the 'Fauna of British India'. After that, extensive work on the diversity and distribution of cerambycids from India is particularly lacking (Majumder et al.,, 2014).

Forty nine species of cerambycids belonging to three subfamilies were recorded from Arunachal Pradesh, India. Subfamily Lamiinae was found to be dominant with 28 species followed by Cerambycinae with 11 species. Subfamily Prioninae included 10 species. *Rhytidodera griseofasciata* reported from China earlier is being reported from India for the first time during the study (Kumawat 2015).

It has been surveyed that 9,00,000 species of insects are described globally in which 6,00,000 species were located and described in India, where many of them are yet to be described or named (Balaji,2016).

Plant species richness, Shannon diversity and the percentage of forest specialists of plants were affected by the degree of urbanization. While the species richness and Shannon diversity of plants value will drease with an increase in degree of urbanization, the percentage of forest specialists was a bit higher in forests located in areas with either a low or high degree of urbanization compared to forests located in areas with a medium degree of urbanization. Additionally, Shannon evenness of plants have a tendency to decrease in forests with increasing percentage cover of sealed areas in their surroundings. (Mellinger, 2018).

Several urban studies have reported a replacement by generalist species of forest specialists with an increase in the degree of urbanization suggesting that forest specialists are more sensitive to urbanization- related disturbances (Deichsel, 2006; Vergnes, 2014; Magura, 2010). This observation was not expected and may be due to the combined effects of differences in habitat diversity in the surroundings, which may be highest at medium levels of urbanization, and of refugia effects of forests in highly urbanised areas (Mellinger, 2018).

Not all species may react to changes in environment which are caused by urbanization in a similar way due to the fact that they have different requirements in regards to their habitat and its surrounding landscape (ConcepcioÂn, 2015; Godefroid, 2007; McIntyre, 2001). An example is that specialist species may respond to the surrounding matrix as a stronger barrier than generalists, which are able to exploit a wider variety of resources from neighbouring green areas (Bai X *et al*., 2008; Croci, 2008). Thus, specialist species become frequently replaced by generalists (Gibb, 2002; Magura, 2004). As a result, species composition in urban

areas becomes progressively similar, which may in turn lead to a decrease in functional diversity homogenisation (LizeÂe, 2011). Additionally, groups of species at higher trophic ranks like herbivores and predators might also be more affected by increased isolation and habitat loss because of their dependence on other species compared to groups of species at low trophic ranks such as plants (Holt, 1999; Steffan-Dewenter, 2003).

Rapid identification of unknown specimens may be achieved through DNA barcoding and query of public sequence databases (Collins and Cruickshank, 2012). This approach has been adopted for use in regulatory fields such as conservation biology, consumer protection, and border biosecurity, especially when morphological identification of target taxa is difficult and/or impossible (Collins *et al*., 2012).

The ability of DNA barcoding to link immature life stages to adult beetles makes it a valuable tool for overcoming the challenge presented by conserved larval morphology (Hendrich *et al*., 2015). However, the application of DNA barcoding depends on the availability of matching reference barcodes within public databases, where the order Coleoptera is still underrepresented compared with Hymenoptera, Diptera, and Lepidoptera (Boykin 2015; Woodcock *et al*., 2013).

OBJECTIVES

The objectives of the present study are as follows:

1. Cataloguing and biodiversity analysis of beetles in Mizoram in relation to the effect of urbanization and forest size.

2. To establish a barcoding system and assess the species composition in relation to urbanization and forest size.

MATERIALS AND METHODS

4.1. Ethics statement

All necessary permits will be obtained from the Chief Conservator of Forests, Department of Environment and Forests, Ministry of Environment and Forests, Mizoram for the field studies. (Permit Number: No.B. 11015/19/2007-FST).

4.2. Study Area

The study was carried out in Aizawl (23.7307° N, 92.7173° E), Mizoram. The study area covers 457 sq. km and located at 1132 m a.s.l. It is located north of the Tropic of Cancer and have a mild to humid sub-tropical climate due to its location and elevation.

Inside the study area, 5 sites were taken into consideration to compare the degree of urbanization between the different areas.These sites were chosen along the urbanization gradient from the urban,i.e, the area lying inside the city, forest or rural area and the semi-urban area which is in between the two.

The study was conducted between October 2018 to September 2019 and sampling was carried out on a bi-monthly basis at the different study sites which are:

A) Mission veng (23° 42’50.56” N, 92 °43’05.86”E)

B) Kanan (23° 42’02.90” N, 92 °43’35.09”E)

C) Sakawrtuichhun (23° 45’40.07” N, 92 °40’15.81”E)

D) MZU, Tanhril (23° 44’26.27” N, 92 °39’23.34”E)

E) PUC, College veng (23° 42’23.73” N, 92 °43’37.99”E)

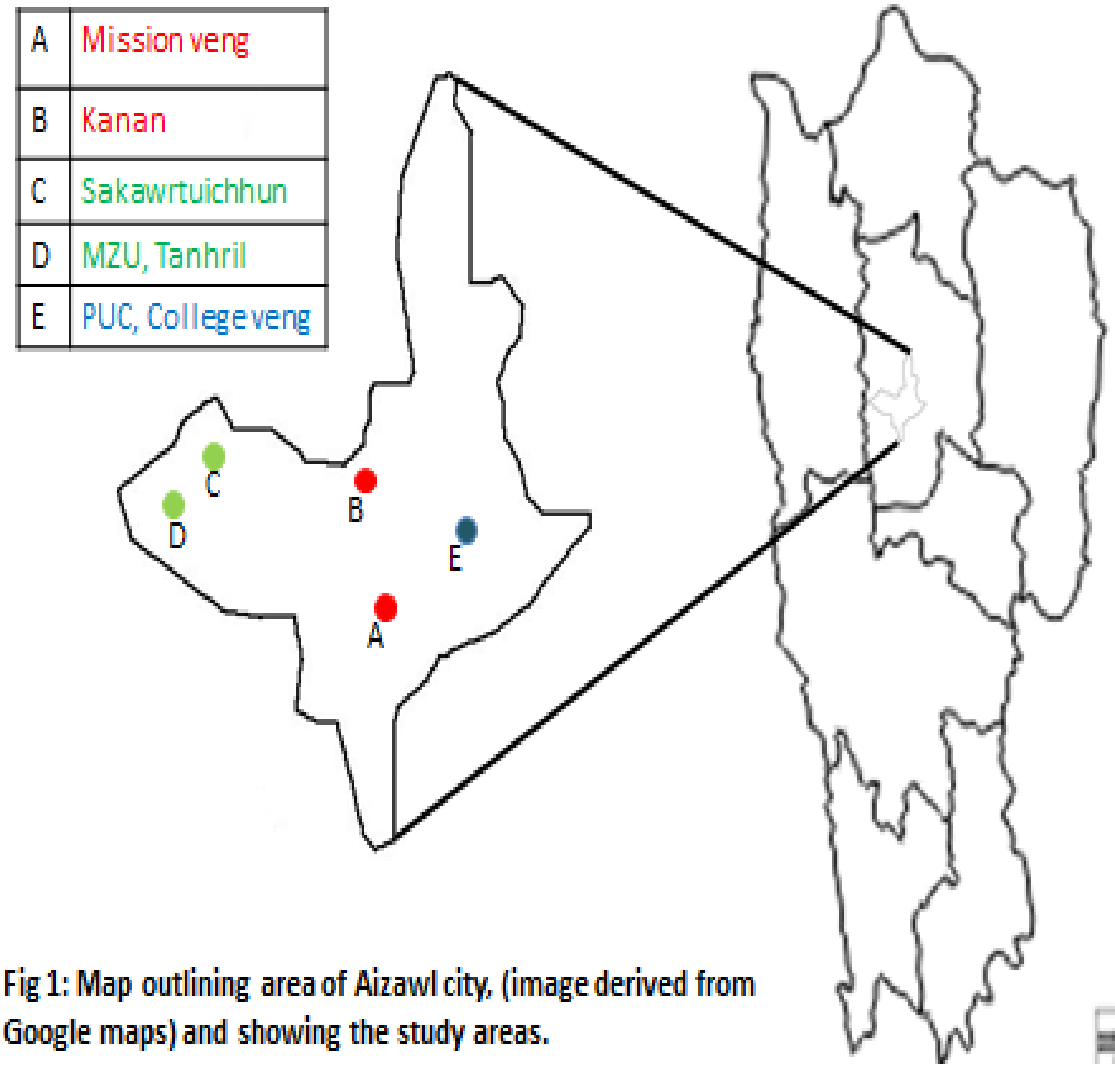

Fig 1: Map outlining area of Aizawl city, (image derived from Google maps) and showing the study areas.

4.3. Sampling of Beetles:

For the sampling of beetles, the methodology as described by White (1998) was primarily followed. Other methods were also used as convenient.

Light trap method was the most dominant method employed during the study for collection of nocturnal species, where lights were placed in the centre of the fields at a height of about 3 meter above the ground and operated between 9:00 PM to 12:00 PM to attract the beetles.

For beetles which are not active at night, manual collection was also carried out by going to the study sites and checking the foliages and trees. Hand picking from the leaves and flowers as well as shaking and beating of the bushes and tree branches to disturb the beetles which were then captured manually or sometimes by using nets for sweeping ground beetles.

The trapped beetles were collected and separated family-wise and the cumulative count was determined at each location. The specimens collected were then stored in 70% alcohol until further use. (Ibrahim *et al*., 2016)

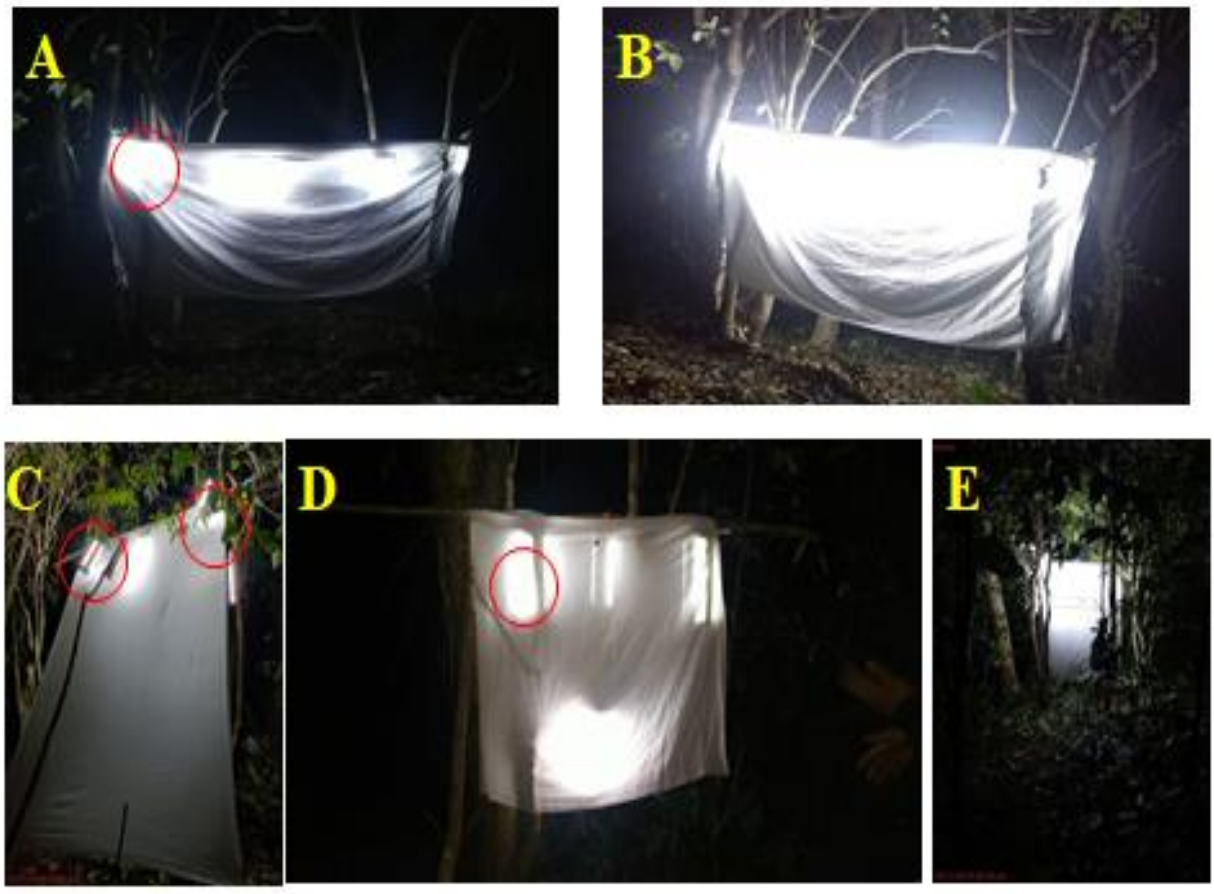

Fig 2: Light traps placed at different study sites (A) Mission veng (B) Kanan veng (C) Sakawrtuichhun (D) MZU campus, Tanhril (E) PUC campus, College veng

4.4. Morphological Identification of Beetles

The identification will be done up to family level using identification keys and by studying the morphological structures of each specimen (White,1998; Castner, 2000).

For the present study, the morphological features like the size and shape of antenna and legs, the shape and colour of the body, etc., were examined and recorded for use in the process of identification. The specimens collected from the study sites were thoroughly examined in the laboratory in order to confirm the initial result and to record the numbers belonging to each family using the identification keys.

4.5. Landscape characteristics and recreational pressure

The urbanization gradient of the sampling sites was determined by the percentage cover of built-up area and traffic infrastructure, urban green space, agricultural land and forest cover, and the percentage cover of sealed area was determined to use as a measure of the degree of urbanisation. (Melliger *et al.*,2018*)*

Land cover data of the landscape characteristics from satellite images (Google Earth, 2009) was derived. Around the most central sampling plot in each area, the percentage cover of built-up area and traffic infrastructure, urban green space, agricultural land and forest cover within radii of 200 m and 500 m was determined using the pixel counting function of Adobe Photoshop (version 10.0.1). (Melliger *et al.*,2018*)*

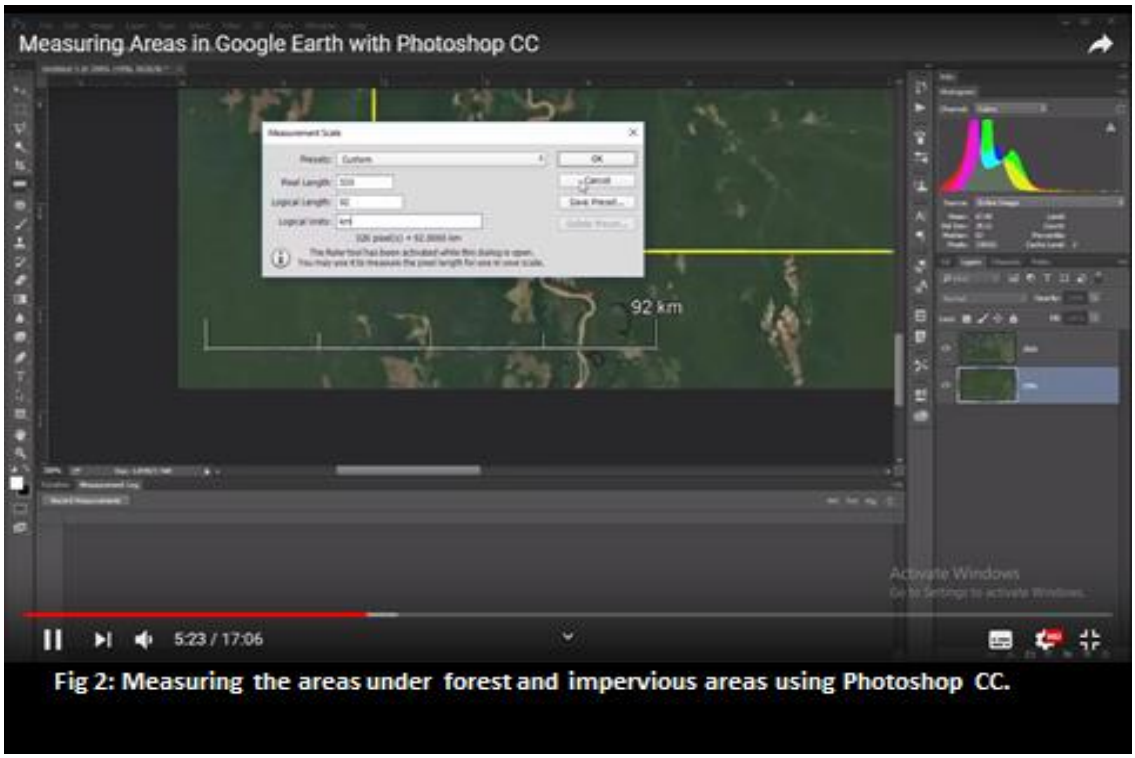

Fig 3: Calculation of percentage cover from Google Earth image using Pixel counting function of Adobe Photoshop

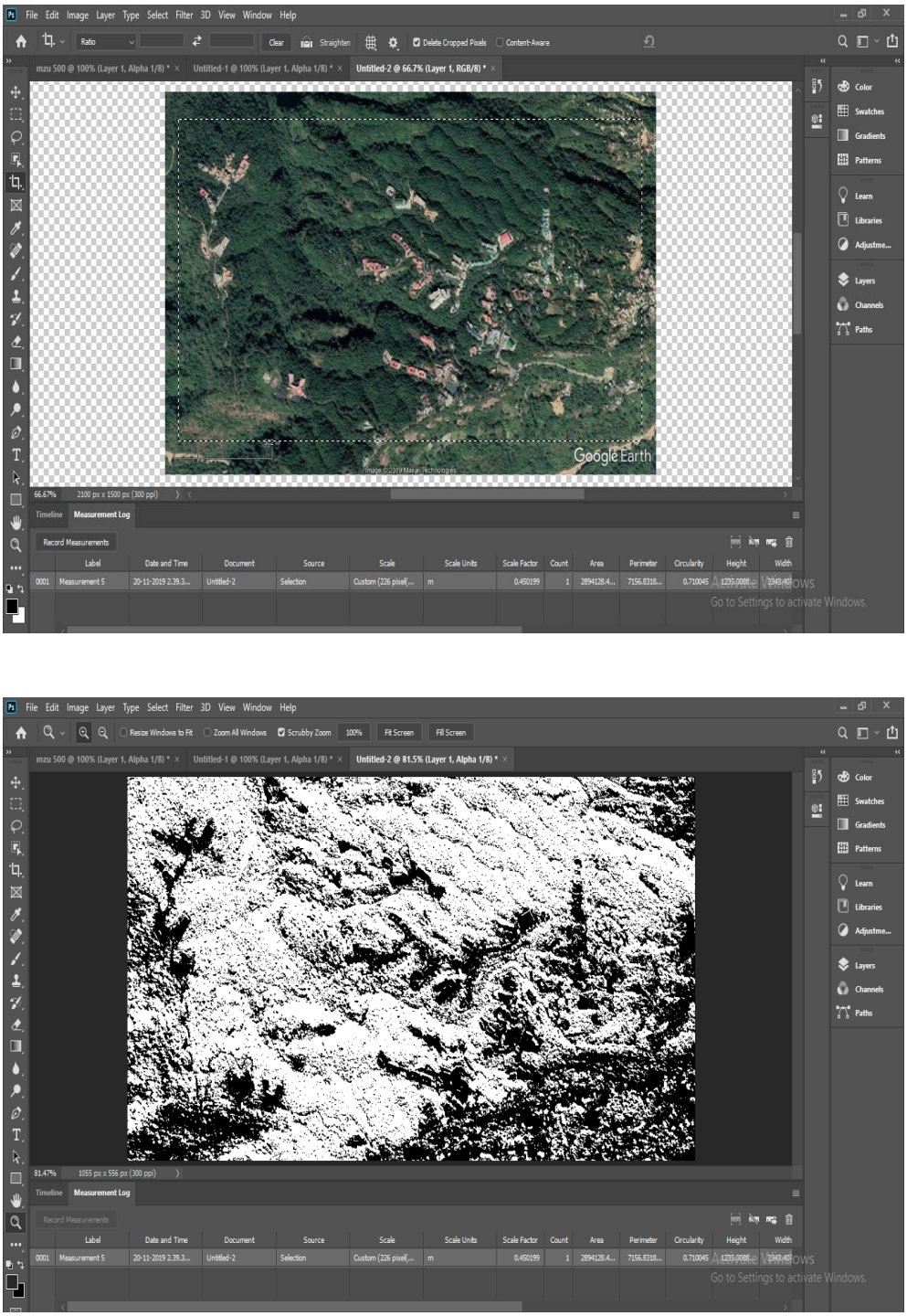

Fig 4: Calculation of percentage cover from Google Earth image using Pixel counting function of Adobe Photoshop

4.6. Diversity Data Analyses:

The following indices were computed:

1. Species diversity indices (i.e., Shannon–Weiner index and Simpson index),
2. Species richness indices (i.e., Margalef index, DM =[(S-1)/ Ln(N)] and Menhinick index),
3. Species evenness and dominance indices (equitability J index or Pielou's evenness index [J'= H'/log S] and Berger–Parker index [d =Nmax /NT]), and

where pi= ni/N, ni represents the number of individuals of species, i and N represents the total number of individuals, S is the number of species, Nmax is total dominant species in a habitat type, and NT is the proportion of the total species (Ibrahim, 2016).

4. Principal component analysis (PCA) was also performed using PAST (version 1.86b) software (Hammer et al., 2001) to investigate and ordinate the relationship between the beetle families' composition and their community association with the habitat. Thus, the axes derived correspond to gradients of species compositional change.

4.7.Genomic DNA extraction

The extraction protocol by Sambrook et al. (1989) was followed. For the DNA extraction, the legs were washed with double distilled water and dried. The legs were macerated with the help of scissors in 1.5 mL Eppendorf tube, and homogenized with pestle, and 250 µL of extraction buffer (100 mM Tris HCl, 200 mM NaCl, 50 mM EDTA, 1 % SDS) was added and mixed gently. Proteinase K (20 mg/mL, 2 µL) was then added followed by incubation in an oven at 56 °C for 30 min. To this, 250 µL of phenol/chloroform (1:1) was added and mixed gently and centrifuged at 13,000 rpm for 5 min. Supernatant was then carefully taken out and collected in a new Eppendorf tube. Absolute ice cold ethanol (450 µL) was added to the supernatant and mixed gently by inverting the tube several times and kept in -20 °C for 30 min. The tube was next centrifuged at 13,000 rpm for 5 min at 4 °C. Ethanol was poured off without dislodging the pellet and 200 µL of 70 % ethanol was added and flash spun at 6000 rpm for 1 min. The ethanol was poured off and the pellet dried. Double distilled water (30 µL) was then added to the tube; and the pellet resuspended by gently flicking the tube and later stored at -20 °C for further use.

4.8. Genome sequencing

The mitochondrial cytochrome c oxidase I (COI) region, which is used in most DNA barcoding analyses of insects, exhibits substantial variation in every one of three nucleotides (i.e., the third codons), even among species in the same insect orders, introducing taxonomic bias into prey community data. PCR was performed

with two universal primers; COI gene: forward primer LepF1 (5′-ATTCAACCAATCATAAAGATATTGG-3′) and reverse primer LepR1 (5′-TAAACTTCTGGATGTCCAAAAAATCA-3′) (Hebert *et al*., 2004). The 25-µL reaction mixture contained 1X amplification buffer, 2.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.2 pM each forward and reverse primer, 0.8 µL BSA, 2 µL genomic DNA, and 1 U Taq DNA polymerase. The PCR thermal regime for amplification used was at 5 min at 95 °C for initial denaturation, followed by 30 cycles of 30 s at 95 °C for denaturation, 40 s for annealing at 51-54 °C, elongation for 30 s at 72 °C, and a final elongation for6 min at 72 °C. PCR products were checked by gel electrophoresis on a 1.5 % agarose gel containing ethidium bromide. Successfully amplified DNA fragments were purified. Samples were then sequenced using Sanger's di-deoxy method, and sequencing reactions were carried out in both directions on a sequencer. All the sequences were checked using BLAST (NCBI).

4.9. Analysis of COI sequences using MEGA 6.0and phylogenetic inference

The sequences of COI from the beetles were aligned using MUSCLE (Edgar, 2004) implemented in the program MEGA 6.0 (default settings retained except maximum number of iterations (maxiters= 1000). Identical sequences were removed. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 6.0 (Tamura *et al*., 2013). Model test (best-fit substitution model) and substitution parameters (test pattern homogeneity, substitution pattern, rate variation among sites, transition/transversion bias, disparity index, amino acid composition, nucleotide composition, codon usage bias, and site-by-site rates) were estimated

using maximum composite likelihood method. Pairwise distances (in over all-, within-, and between-group and net between group mean distances), diversity (in entire population, mean inter-population, and coefficient of differentiation), bootstrap, and analytical variances were also computed. Computed distances were separated based on site degeneracy, codon sites, transitions and transversion bias, and non-synonymous and synonymous changes. Codon-based Z test and Fisher's exact test of selection and Tajima's test of neutrality were performed. Gamma parameter for site rates, position by position rates, and Tajima's relative rate test were done. The analysis involved 11 nucleotide sequences of COI gene from beetles. Codon positions included were first + second + third+ non coding. All positions containing gaps and missing data were eliminated (Tamura *et al*., 2013). The ratio of the number of non-synonymous nucleotide substitutions per site (dN) to that of synonymous nucleotide substitutions (dS) based on a set of aligned COI sequences were performed to investigate the selection pressure among the beetles COI gene by using online tool SNAP v2.1.1 (Korber, 2000). Phylogenetic relationships were inferred using Maximum Parsimony (MP) for COI datasets. MP trees were obtained using PAUP 4.0b10 (Swofford, 2002) by heuristic search option with tree-bisection-reconnection (TBR) branch-swapping. The number of bootstrap replicates was set at 1000.

4.10. Sequence statistics and identification success

BOLD tool was used to calculate the nucleotide composition of the sequences and distributions of Kimura-2-Parameter distances within and between species. The

performance of barcode sequences in species identification was assessed by conducting a barcode gap analysis in BOLD. All species found to share haplotypes with one or more other species were interpreted as identification failures.

4.11. Barcode Index Numbers

The Barcode Index Number (BIN) system were created as an interim taxonomic system to aid management of the 3 M barcode sequences in BOLD. Sequences were assigned to BINs using the Refined Single Linkage (RESL) algorithm which performed an initial single linkage analysis employing 2.2% sequence divergence as a minimum distance between clusters. The resulting operational taxonomic unit (OTU) boundaries were then refined by Markov clustering. The BIN assignments on BOLD are constantly update as new sequences were added, and individual BINs were split or merged in light of new data. The BIN assignments used in this study were downloaded from BOLD. Ratnasingham and Hebert (2013) scheme was used for comparison to examine the correspondence between traditionally recognized species and the OTUs delimited by the RESL algorithm. Each species was assigned to one of four categories as follows: (1) Match: all specimens of a species included in one BIN; (2) Split: specimens of a single species divided into two or more BINs; (3) Merge: all specimens of two or more species combined into a single BIN; (4) Mixture: Both a merge and a split involving two or more species.

4.12. Soil analysis:

Top soil samples (0–10 cm) were randomly collected from each sampling site, and stored in plastic bags. The composite soil samples were used for soil analyses. Soil pH, organic carbon, available phosphorus, sulphur and total nitrogen were measured following the standard protocols (Anderson and Ingram 1993; Black *et al.,* 1965). Evaluation to assess the correlation between soil health and diversity of beetle families was also done using PAST 3.25 Software.

4.13. Vegetation analysis

The analysis of vegetation was applied based on line transect method of 50 x 50 m^2 of five quadrates analyzed (5x50m = 250m^2) with five sub-plots each at four corners (5x10 = 50 m^2). All trees >20 cm in diameter at breast height (DBH) shrub, climber, vines and lianas >5 cm were measured and identified within each plot. Apart from tree density, the vegetation was also analyzed for relative frequency, relative density, relative dominance and species IVI (Mishra, 1989; IJSC, 2015).

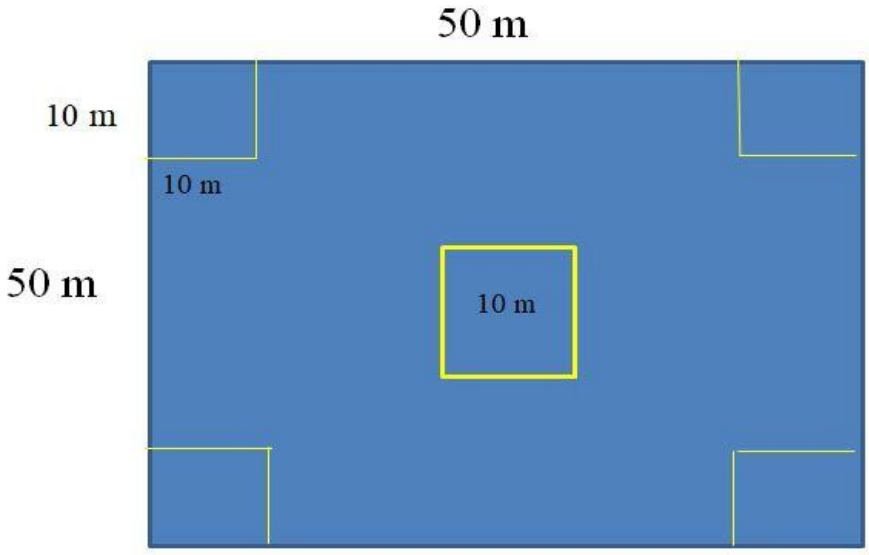

Fig 5: Quadrat with sub-plot

RESULTS

5.1. Biodiversity of Beetles

During the study period of 12 months, a total of 445 beetles belonging to 15 families were collected from all the sites taken together. From the results obtained, it was observed that the most number of beetles was collected from MZU campus which has a considerable forest cover and hence taken as the forest or rural site, followed by Sakawrtuichhun which is also a rural site. The number of beetles collected from Mission veng and Kanan veng were the least. On one hand, PUC campus showed a surprisingly less number of beetles collected from the site despite it being a semi-urban site.

Out of the 15 families recorded, the total number was recorded from the Cerambycidae family which could be found on all the 5 different sites followed by Lucanidae and Scarabidae. The family Buprestidae, Lampyridae and Meloidae were regarded as habitat specialists as they were found only is certain habitats. There are also some specific species from other families which are highly specialized in their habitat while some others from the same family were not found to be so as in the case of Chrysomelidae and Curculionidae.

Further, it was also observed that the collection of beetles was most successful during the spring and summer seasons (late March to early July) as compared to the winter season (October-January), while the rainy season (towards end of July to October) are good collection period for only few selective species and the more common generalists.

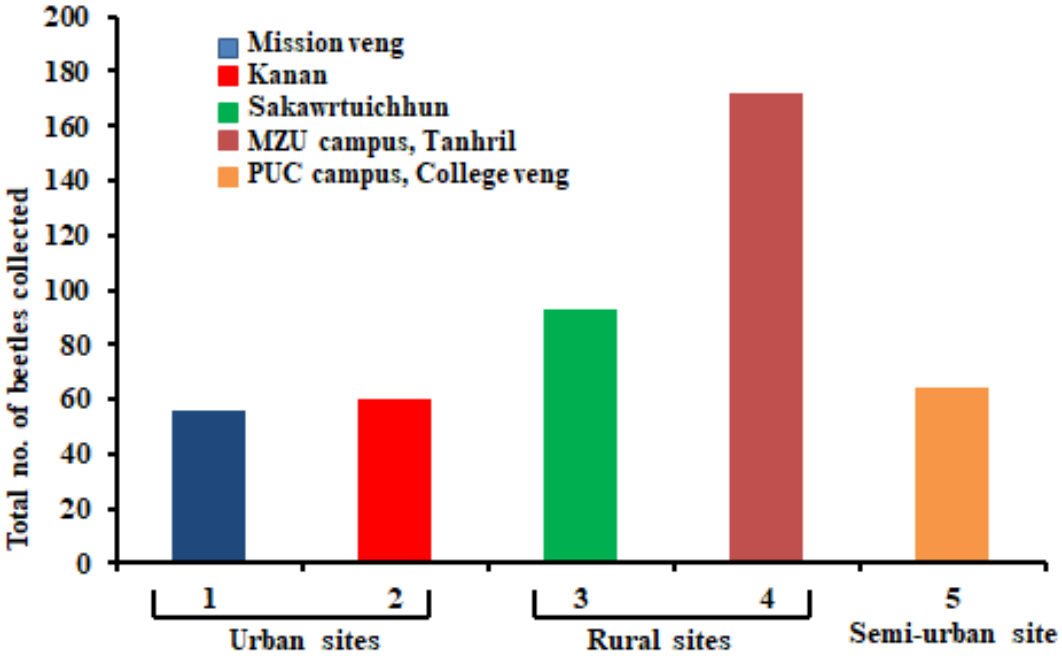

Fig 6: Graph showing the total number of beetles collected from the different sites

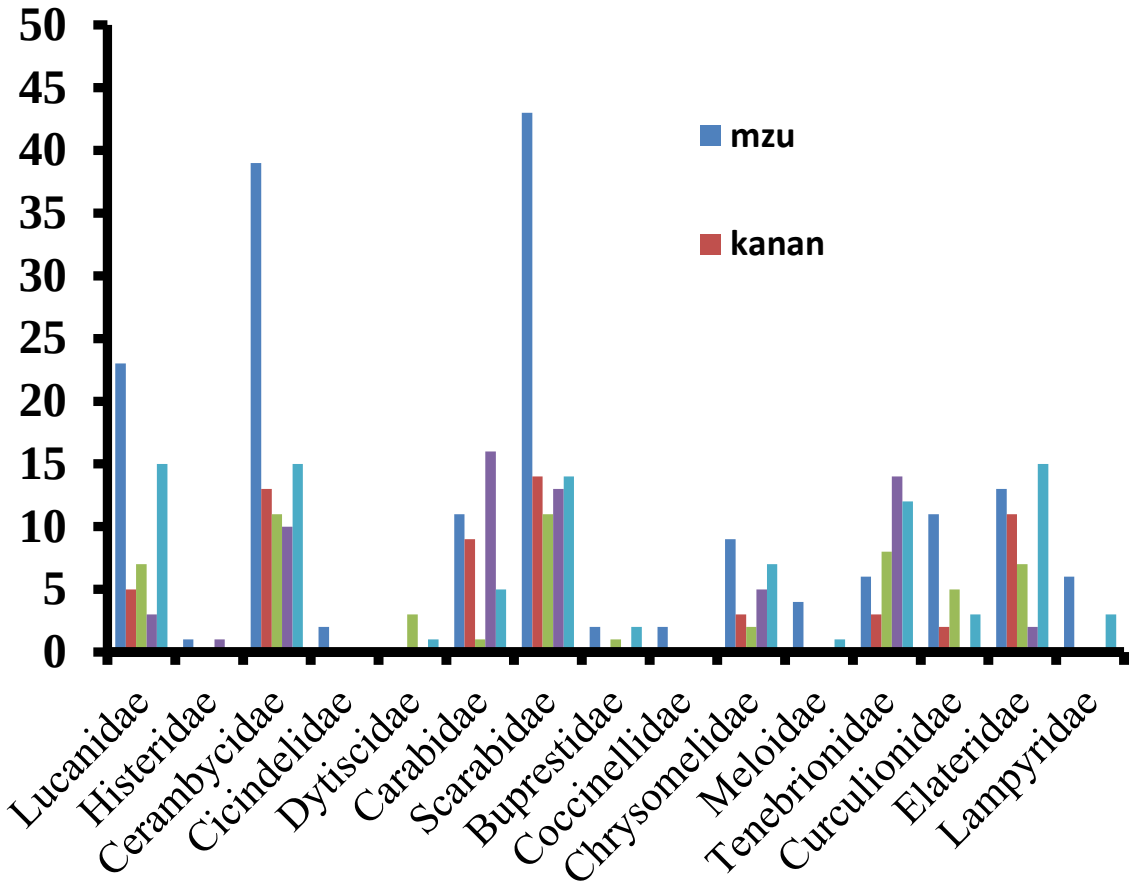

Fig 7: Graph showing the distribution of total number of beetles in each site

Soil and vegetation analyses were also taken which were then correlated with the data from the diversity of beetles to give the relation between the degree of urbanization and forest size in all the study sites.

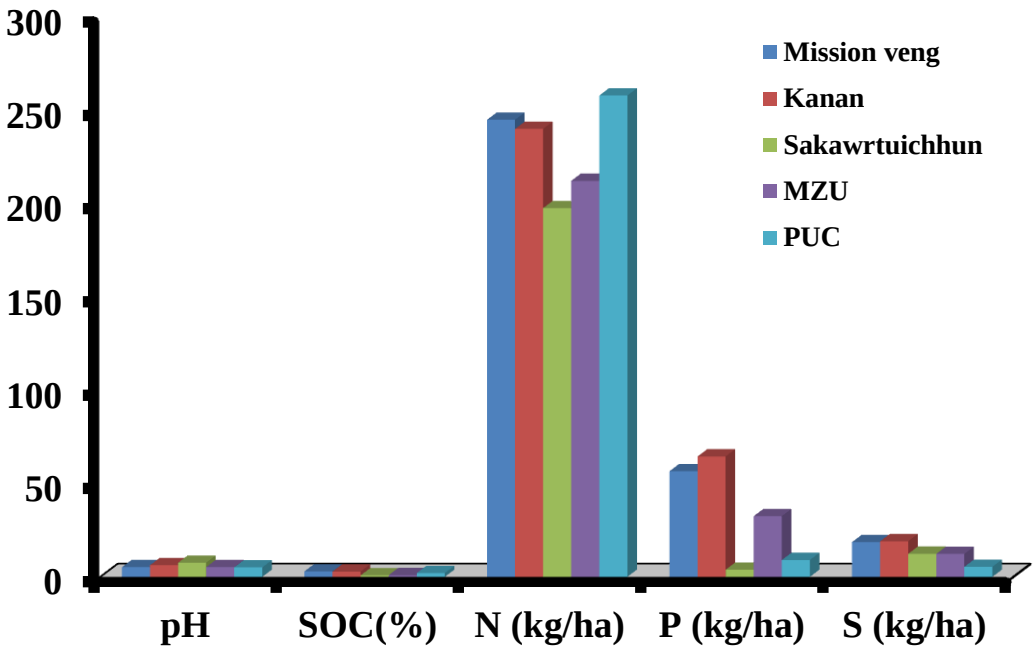

Fig 8: Results of soil analysis from the different study sites

5.2. Identification keys and features of collected beetle families

Following are the list of the 15 families to which the collected specimens belong to, along with a few basic keys which are used for indentification of the families.

1. **Buprestidae or Jewel Beetles**
    - The body is bullet shaped often with metallic sheen.
    - The elytra is hard and shell like and come together posteriorly in a blunt point.
    - The antenna is saw-toothed or thread like.
2. **Carabidae or Ground Beetles**
    - The head and eyes as wide or wider than the protonum.
    - The elytra is seldom patterned but usually have ridges or rows of punctuations.
3. **Cerambycidae or Long-horned Beetles**
    - The antenna is at least half their body length and may even be upto twice their body length.
    - The general body shape is elongated and cylindrical and having tarsal formula of 5-5-5 although it may seem as 4-4-5.
4. **Chrysomelidae or Leaf Beetles**
    - The antenna is short and usually less than half the body length.
    - The third tarsal segment is bi-lobed.
    - The body shape is variable from rounded to broadly oval to pear-shaped.

5. **Cicindelidae or Tiger Beetles**
    - The eyes are large and bulbous and the width of the head and the eyes as wide r wider than the protonum.
    - The mandibles are large and toothed and the antennae originate from above the base of the mandibles.
    - The elytra are often patterned but usually without ridges or rows of punctuations.
6. **Coccinellidae or Ladybugs**
    - The body is usually rounded and extremely convex.
    - They are usually brightly colored or spotted red, orange or black.
    - The head is partially concealed by the protonum.
7. **Curculionidae or Weevils or Snout Beetles**
    - The head is with a well-developed "snout" ranging from short and broad to long slender and curving.
    - The antenna arises at the start of the snout and is elbowed with the last 3 segments forming a club.
8. **Dytiscidae or Predaceous Diving Beetles**
    - The hind legs are long, flattened and fringed with hairs.
    - The body is convex and oval in shape.
    - The scutellum is small but usually visible.
9. **Elateridae or Click Beetles**
    - The body is elongated with the elytra narrowing posteriorly.
    - The posterior corners of the protonum are pointed.
    - Backward pointing spine on venter, originating between the front pairs of legs and fitting into the grove between the middle pair of legs.

10. Histeridae or Clown Beetles

- The elytra is short and squared off at the tips, exposing the tip of the abdomen usually 1-3 segments.
- The antenna is short and clubbed.

11. Lampyridae or Fireflies

- The body is elongated and parallel-sided.
- The head looks concealed by the protonum but not concealed when viewed ventrally.
- The elytra are leathery and tip of abdomen is yellow or white when viewed ventrally.

12. Lucanidae or Stag Beetles

- The mandibles are modified into large antlers especially in males.
- The antennae are elbowed with last 3-4 segments forming a club that cannot be held tightly together.
- The protonum is separated from the elytra.

13. Meloidae or Blister Beetles

- The protonum is narrower than the head and the base of the elytra.
- The elytra is soft and leathery and curving loosely around the body sometimes exposing the tip of the abdomen.

14. Scarabidae or Scarabs

- The family consists of robust or bulky convex bodied beetles.
- The antennae are lamellated with last 3-7 segments composed of flattened lobes that can be held together.
- Rhino beetles and Dung beetles are present under this family

15. Tenebrionidae or Darkling Beetles

- The margin of the eye is notched by kneel-like ridge on the head.
- The protonum is as wide as the body.
- The elytra are hard and shell-like.

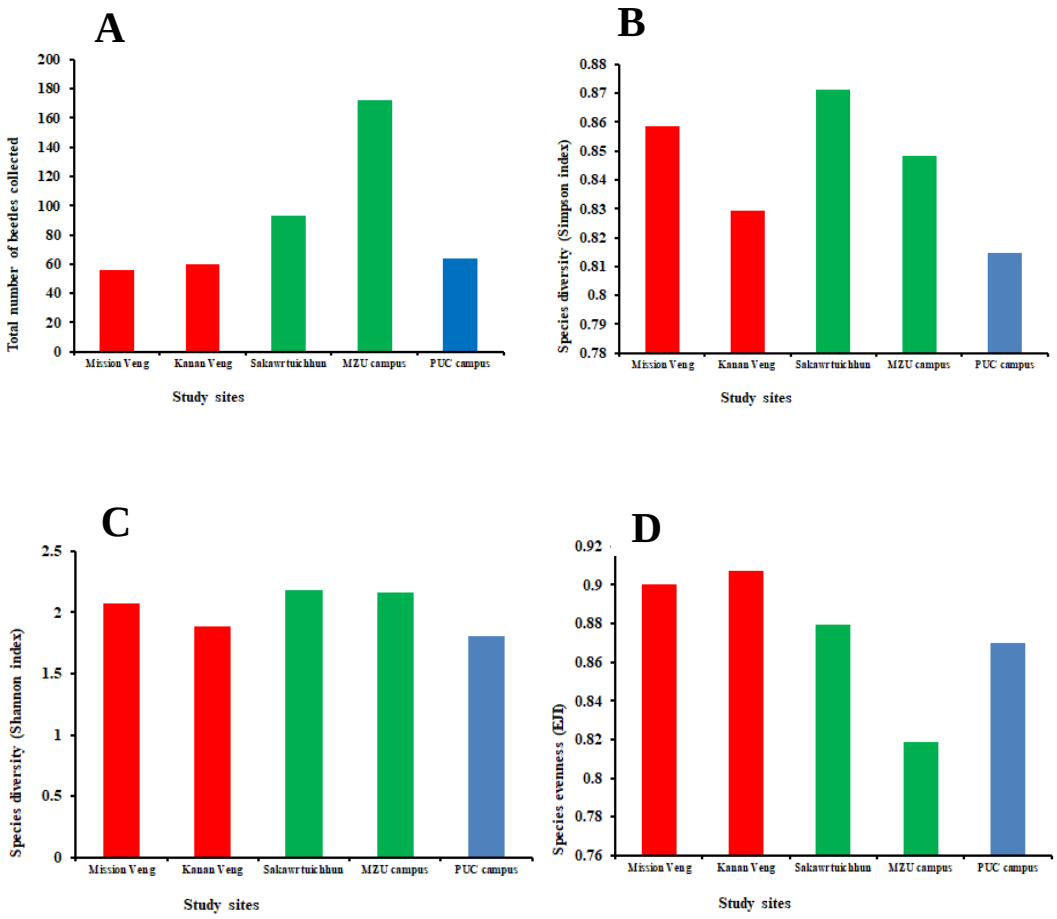

Fig.9: Assessment of beetle species diversity indices (A) The total number of beetles captured. (B) Species diversity index [Simpson index]. (C) Species diversity index [Shannon–Weiner index (D) Species evenness [equitability J index (EJI)].

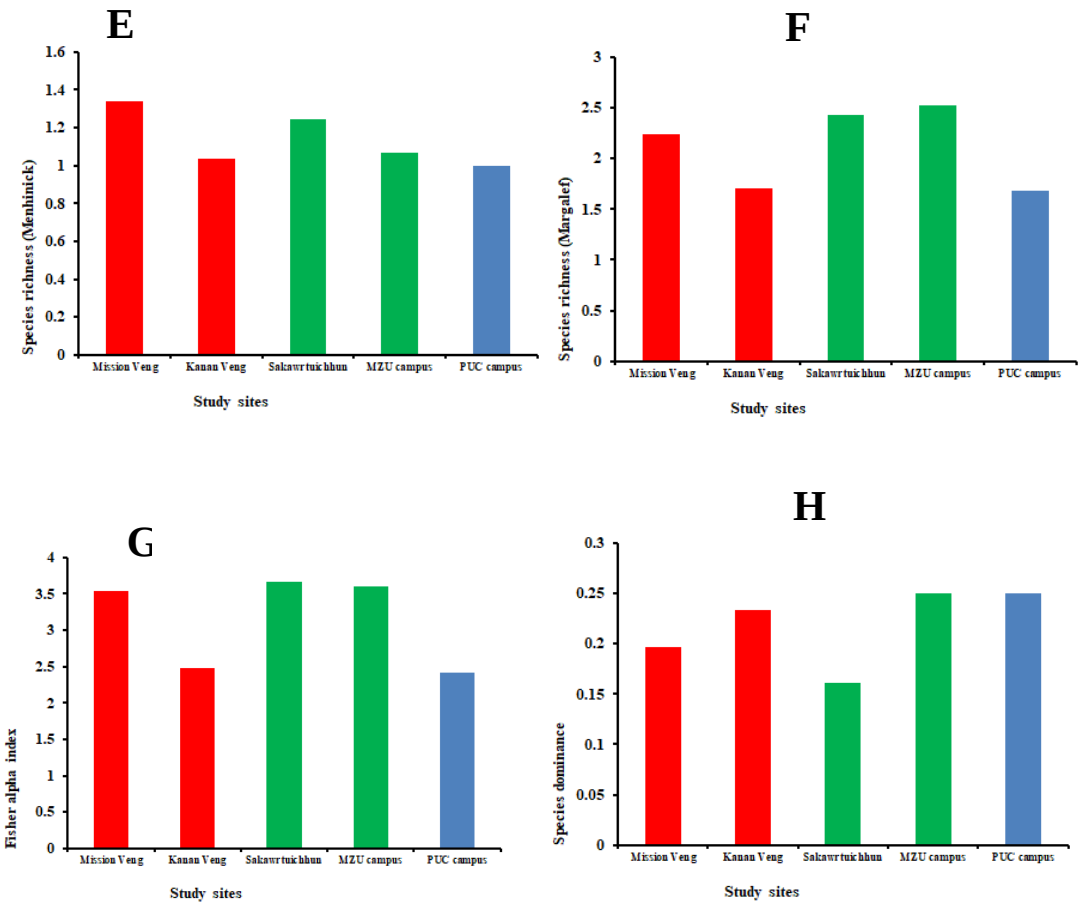

Fig 10: Assessment of beetle species diversity indices. (E) Species richness index [Menhinick index]. (F) Species richness index [Margalef index]. (G) Fisher alpha index. (H) Species dominance indices [Berger– Parker index (BPI)].

5.3. Biodiversity Indices

From the figs. 9 and 10, we can infer that the total number of beetles collected was most from rural or the forest sites while it is the least from the urban sites. The PUC campus which is situated within the city boundary but is equipped with a considerable amount of vegetation cover and large trees was taken as the intersection area or semi-urban area.

The Simpson index which is a diversity index showed higher values in the rural than in urban sites which is directly complemented by the values in Shannon diversity index. Not surprisingly, the semi-urban site once again showed the lowest values in both. This values are in agreement with the previous said result shown by the total number of individual specimens collected from all the different sites, not taking into account the families.

Then we have the species evenness in equitibilty J index which gives us how close in numbers each species, or in this case, the families are in an environment. Here, the graph showed the lowest in MZU campus, while it is high in Mission veng and Kanan.

From the Menhinick index, we can see that all sites are more or less equal with only Mission veng showing a little bit higher value than the rest. From this we can say that this slightly higher value will mean that the site contains the most number in terms of species count, not taking into account the abundance of the species. Maragalef index on the other hand, while showing more or less the same, the only

difference being that instead of the forst site, now we have the MZU campus with the most value.

The Fisher alpha index gives us Kanan veng and PUC with lower values from which we can infer the richness of species in these two sites and once again this gives true when compared with the Margalef index values. Lastly, the dominance is found to be highest in the MZU campus and this is much expected as well, while although having a high total individual number but is low in dominance for Sakawrtuichhun site. Then again, Kanan veng which was not having high number of individuals is surprisingly high in dominance of species. This may be attributed to the fact that the site has a high number of generalist species.

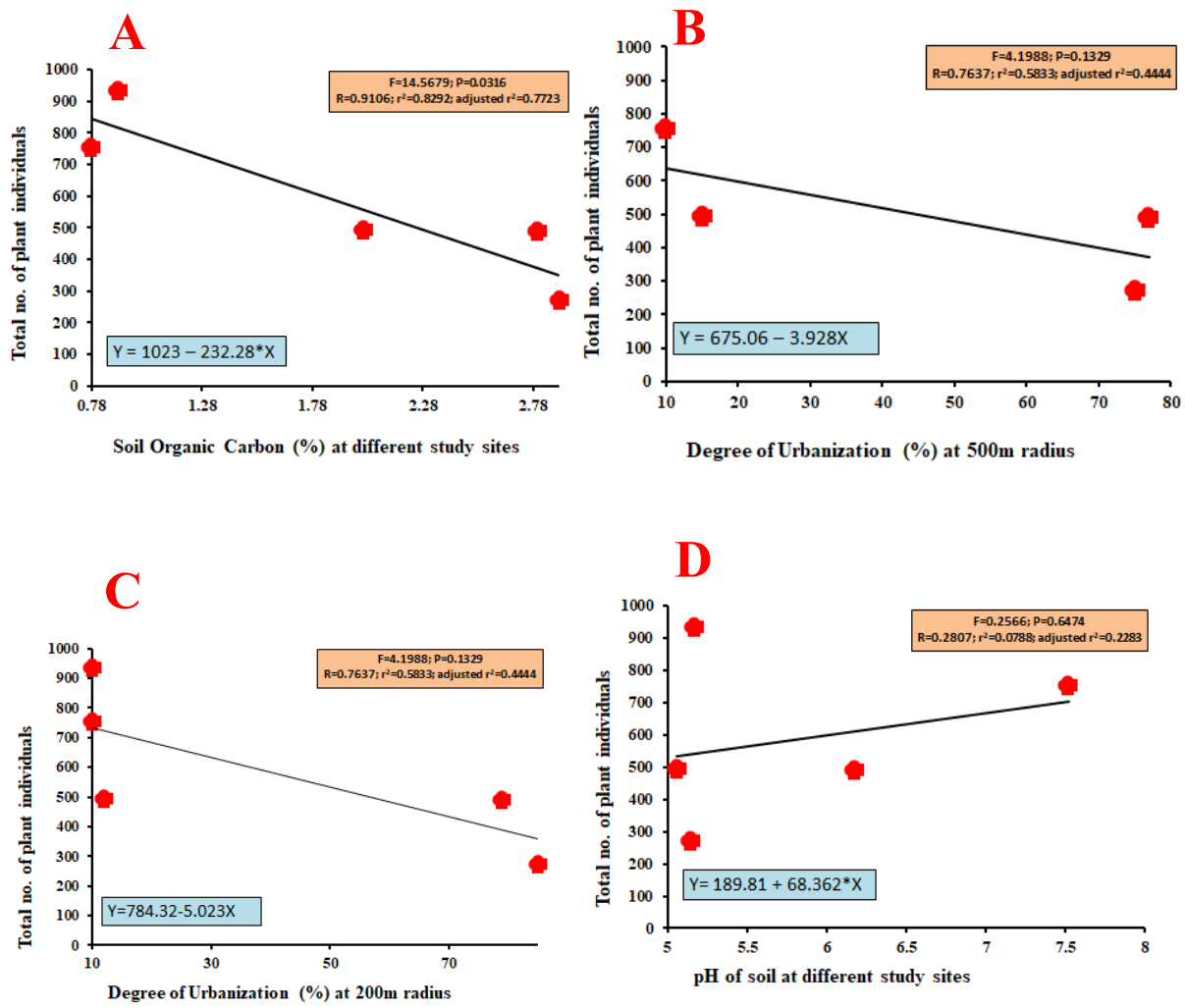

Fig 11: (A) Correlation between degree of urbanization in study sites and total no. of plants at 500 m radius
(B) Correlation between degree of urbanization in study sites and total no. of plants at 200 m radius Total Sulphur
(C) Correlation between total no. of plants and soil organic carbon at different study sites
(D) Correlation between total no. of plants and soil pH at different study sites

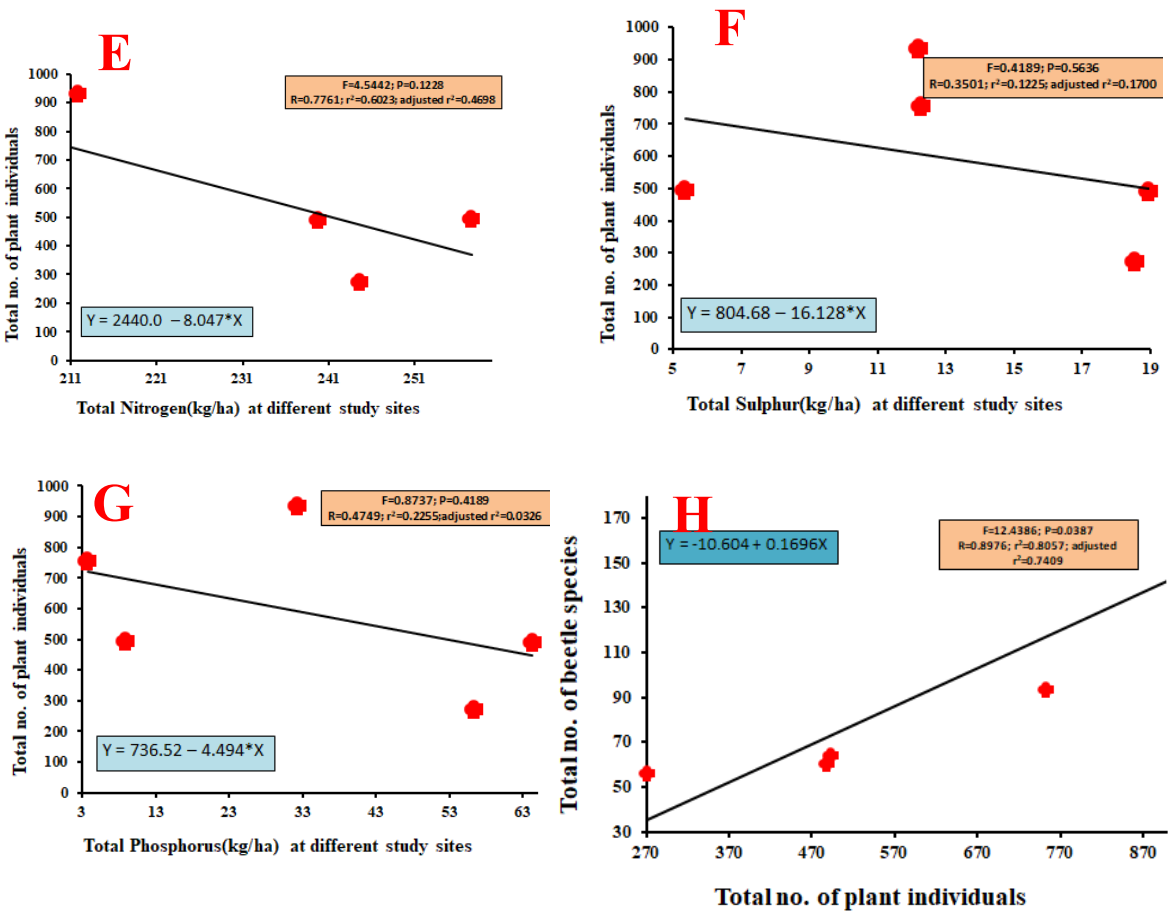

Fig.12: Correlation between the total no. of plant individuals and (E) Total Soil Nitrogen
(F) Total Sulphur (G) Total Phosphorus at different study sites
(H) Correlation between the total no. of beetles collected and total no. of plant individuals

5.4. Correlation between Soil-Plant-Beetles

On studying figs. 11 and 12, from the fig A, we can see the correlation between soil organic carbon and the total number of plants in the different study sites which is showing a negative correlation. This means that with the increase the soil prganic carbon, the plant number is actually decreasing at each site. Then in (B)&(C) of fig.11, we can see the correlation between the total number of plants in the different study sites with the degree of urbanization at 500m and 200m radius which is both showing negative correlation between them meaning that with the increase in urbanization in an area, the plant or vegetation will be decreasing respectively. Fig 11. (D) is showing a positive correlation between soil pH and total number of plant individuals in each sites.

Then on looking at fig 12., the first three are showing correlation between the different soil parameters and total number of plant individuals in the different sites. The increase in the levels of soil Nitrogen, Sulphur and Phosphorus are all surprisingly showing a negative correlation which means the plant individuals will actually be decreasing.

Finally, after the correlation between first the plants with urbanization and plants with soil, we can also correlate the plant individuals with the beetle population. The scatter diagram showed a positive correlation between the two which means that in the situation or site with increasing number of plants will have an increase in the beetles population as well.

5.5. Genomic Sequencing and DNA Barcoding of beetles

The collected specimens were taken and after segregating into different families, the representative species from all families was taken for DNA extraction. A total of 82 beetles were taken for DNA extraction. This number includes some which are same species. After checking the results, DNA was extracted successfully from 76 samples and then this was taken for quantification using PCR. The PCR products were taken for sequencing and the sequences were eventually taken for a series of phylogenetic analyses and constructing phylogenetic trees using Maximum likelihood method, Minimum evolution method, Maximum parsimony, Neighbour joining and UPGMA methods in order to find the distance of relationship between the different beetle families sampled. The constructed phylogenetic trees can be observed in figures 13-17.

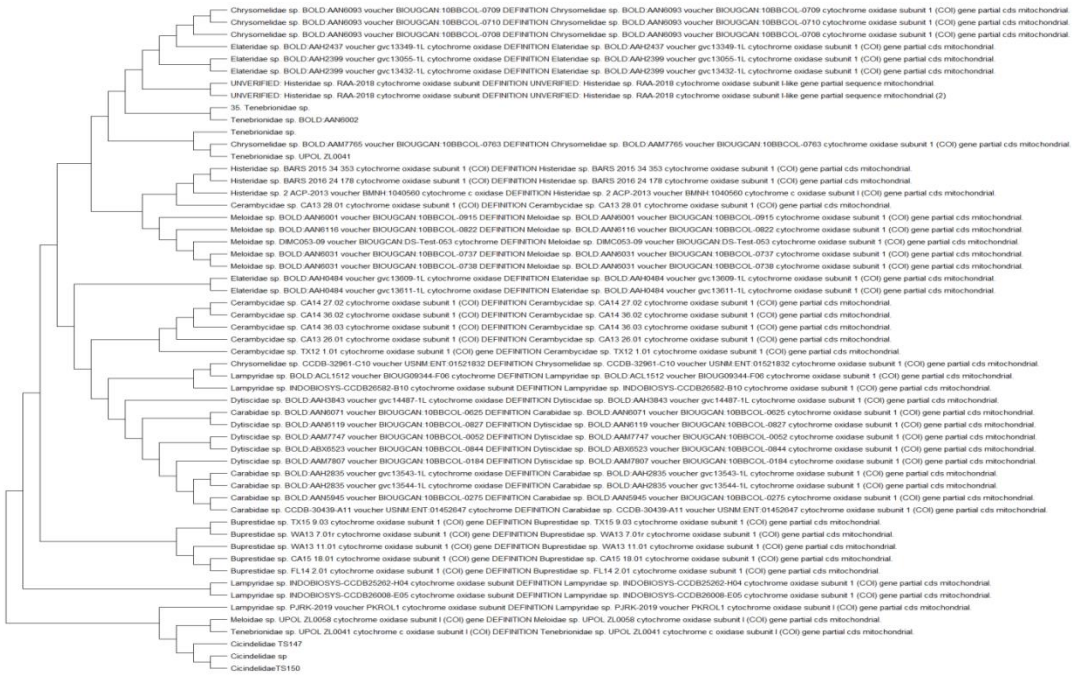

Fig 13: Maximum likelihood tree of the families of beetles sampled

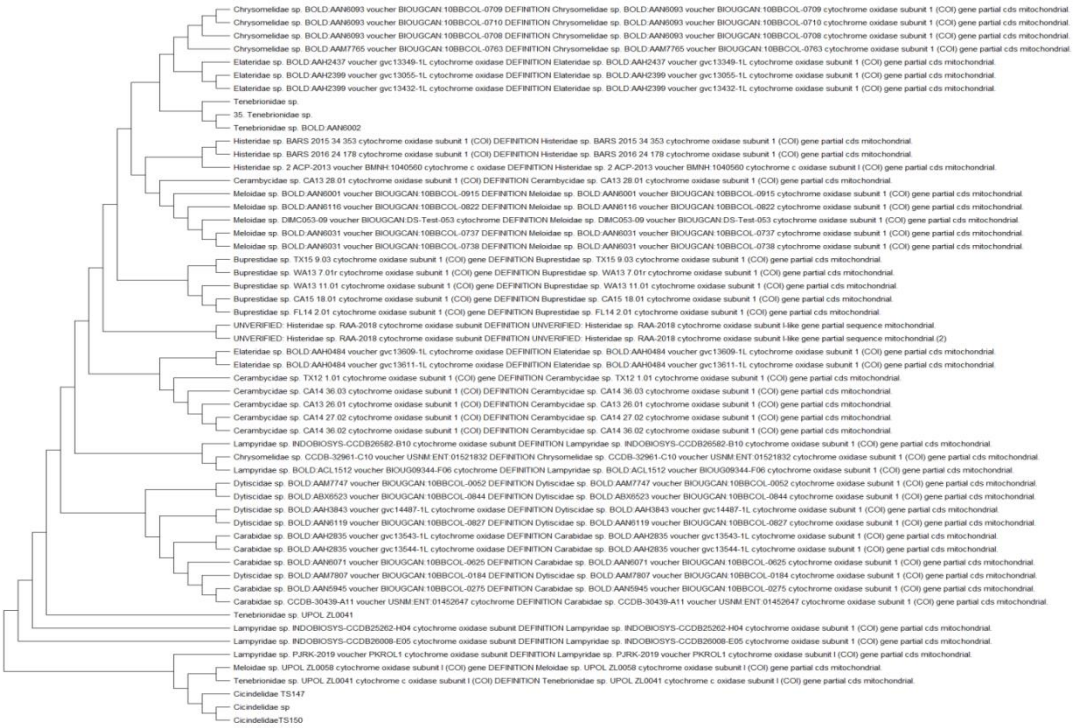

Fig 14: MInimun evolution tree of the families of beetles sampled

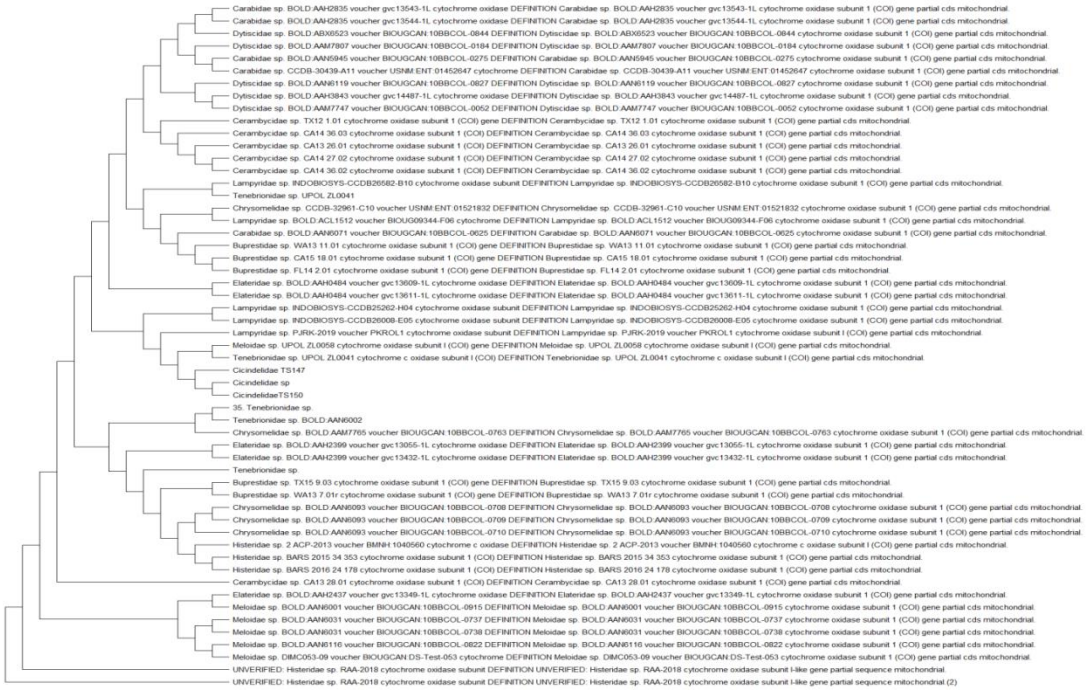

Fig 15: Maximum parsimony tree of the families of beetles sampled

Fig 16: Neighbour joining tree of the families of beetles sampled

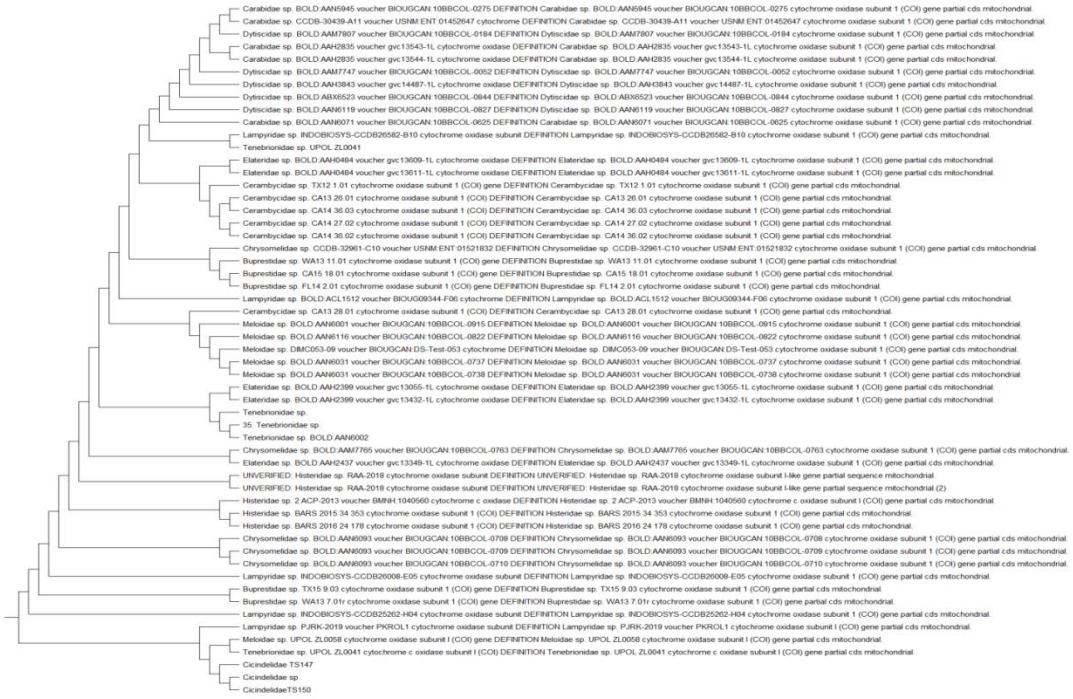

Fig 17: UPGMA tree of the families of beetles sampled

DISCUSSION

AND

SUMMARY

Beetles are the largest in numbers in all the orders under the kingdom Animalia. Although they may be small, they are extremely diverse in terms of expediency as well as incompetence. While there are many under this order which are beneficial to mankind as a means of bioindicators (Filgueiras *et al.,* 2011) or as environmental impact assessments (Bicknell *et al.,* 2014), especially the oxynelid species. There are also some serious pests among the beetle families, for examples, desmestids are serious house pests and wood boring beetles are pests of plants and lumber.

In the study the diverse effects of the degrees of urbanization and forest size on beetles diversity and species composition was conducted where all the different factors and parameters were taken into account. Urbanization is increasing at a steady rate in every places, even in our own city of Aizawl as compared to a decade ago or even during the last few years. With increase in urbanization comes the decrease in forest size due to agricultural purposes, for building infrastructures and roadways, and many other reasons. This has led to a disturbance in the ecosystems which were present initially at the different locations. This has also led to ecological imbalance and can also be attributed to anthropogenic activities and climate change.

While there may not have been so much scientific records to study the local beetles community and taxonomy, from the local records by the elders of tribal population we have come to know that some species which were known to be very much dominant in earlier years have now been in decline while some new species are emerging. They are mostly the specialists species which could not endure and the

generalists species which thrived. There are also new species which might either be exotic species or even new species.

The study has shown that the beetles diversity is very much affected by the degrees of urbanization and local forest size where with increase in urbanization comes the decrease in forest size and subsequently the decrease in beetles diversity as well as in species composition as well. The soil parameters also play an important role in that the soil directly affects the presence and growth of plants in the areas, and these plants play host to the diverse assemblages of beetle population. The soil chemistry also plays an important role in that some adult beetles and most eggs and larval stages are known to reside in the soil itself. This is true especially in the case of the Carabids and Cicinelids. Some other families like the leaf beetles, blister beetles and wood boring beetles are also primarily found on the leafs and on trunks of trees.

The present study although we obtained considerable results, is not sufficient enough due to a number of reasons, one being time constraint. So in order to get a more accurate and detailed analysis of beetles biodiversity, it is suggested to extend the study period and over a larger and more well defined area. In this study, the functional traits and environmental factors were not taken in consideration, but is suggested that they be done in case of further studies if possible. The climate in each season also plays a factor in the distribution of the beetles population in the study areas where the different niches are found to be loved by the different assemblages of beetles which is not that surprising considering the diversity.

On the other hand, while diversity of beetles and various indices were analyses critically, taxonomic approaches which were also carried out gives us a more effiecient result in finding out the taxonomy. But this can also be a problem when it is a pioneer work with little or no prior work done on it and there may not be the sequences which could be referred for comparison. The phylogenetic analyses of the beetle families is also very useful in finding out the distance of relation between not only two families but even within same family, we can check if there are closer relationship between two or more specific species.

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PLATE 1

Cerambycidae (Long-horn Beetles)

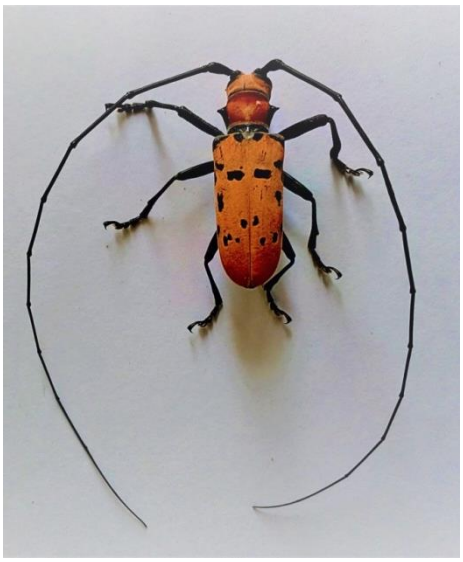

PLATE 2

Cerambycidae (Long-horn Beetles)

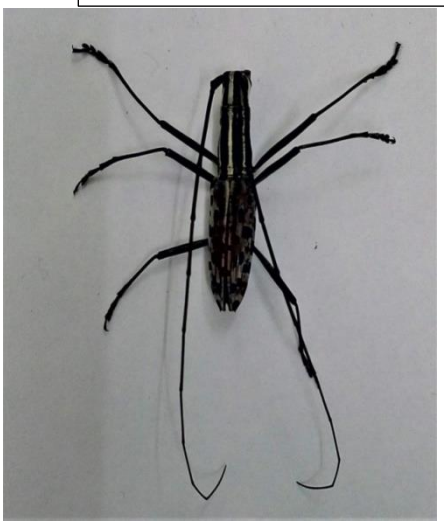

PLATE 3

Cerambycidae (Long-horn Beetles)

PLATE 4

Cerambycidae (Long-horn Beetles)

PLATE 5

Cerambycidae (Long-horn Beetles)

PLATE 6

Cerambycidae (Long-horn Beetles)

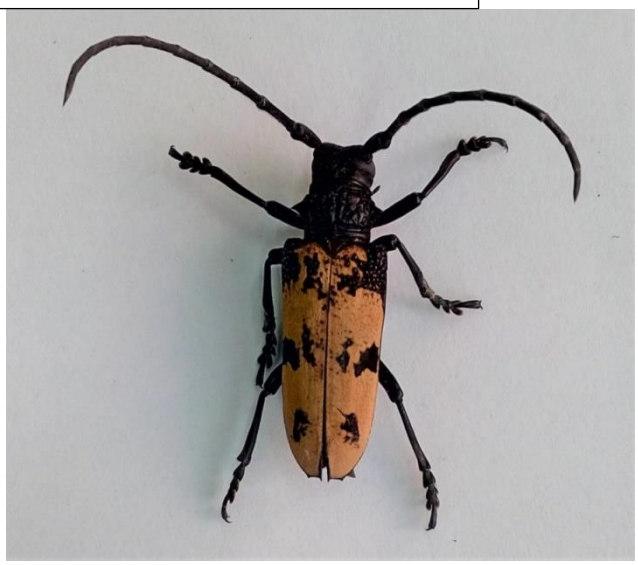

PLATE 7

Cerambycidae (Long-horn Beetles)

PLATE 8

Lucanidae (Stag Beetles)

PLATE 9

Lucanidae (Stag Beetles)

PLATE 10

Buprestidae (Jewel Beetles)

Meloidae (Blister Beetles)

PLATE 11

Scarabidae (Scarab Beetles)








PLATE 12

Chrysomelidae (Leaf Beetles)







PLATE 13

Carabidae (Ground Beetles)

Tenebrionidae (Darkling Beetles)

PLATE 14

Curculionidae (Snout Beetles)

Cicinelidae (Tiger Beetles)

PLATE 15

Elateridae (Click Beetles)



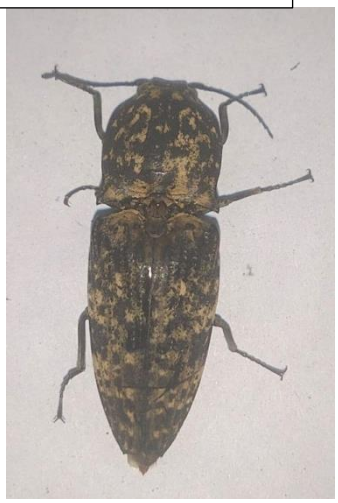


PLATE 16

Lampyridae (Fireflies)

Coccinelidae (Ladybugs)

Dytiscidae (Diving Beetles)

Hiteridae (Clown Beetles)

PARTICULARS OF THE CANDIDATE

NAME OF THE CANDIDATE	:	MALSAWMDAWNGZUALI TARA
DEGREE	:	MASTER OF PHILOSOPHY
DEPARTMENT	:	ZOOLOGY
TITLE OF THESIS	:	DIVERSE EFFECTS OF DEGREE OF URBANIZATION AND FOREST SIZE ON BEETLES BIODIVERSITY AND SPECIES COMPOSITION IN AIZAWL, MIZORAM
DATE OF ADMISSION	:	01.08.2018

APPROVAL OF RESEARCH PROPOSAL

1. BOS	:	08.04.2019
2. SCHOOL BOARD	:	17.05.2019

REGISTRATION NO. & DATE	:	MZU/M.Phil/531 of 17.05.2019

HEAD
Department of Zoology

LIST OF CONFERENCE/SEMINAR/WORKSHOP ATTENDED AND PARTICIPATED

- "National Seminar on Conservation and Sustainable Use of Medicinal and Aromatic Plants" Organized by Department of Forestry, Mizoram University, Aizawl, Mizoram.
- The 12th Annual Conference of Association of Biotechnology and Pharmacy (ABAP) & International Conference on Biodiversity, Environment and Human Health: Innovations and Emerging Trends (BEHIET 2018)
  Organized by School of Life Sciences, Mizoram University, Aizawl, Mizoram and ABAP, India.

- The International Conference on Chemistry and Environmental Sustainibility (ICCES-2019)
  Organized by Department of Chemistry, Mizoram University, Aizawl, Mizoram.

- Two-days Training Programme on "Understanding the Impact of Forest Fire on the
  Faunal Resources of North-Eastern States"
  Organized by Zoological Survey of India, Kolkata.

- National workshop on "'Bioinformatics for Zoologists'
  Organized by Bioinformatics Infrastructure Faculty (BIF), Department of Biotechnology, Mizoram University, Aizawl, Mizoram.

- International Conference on Chemistry and Environmental Sustainability (ICCES-2019) held during 12-22 February,2019 by the Department of Chemistry, Mizoram University.

- NRDMS-DST Orientation Program on Geospatial Technologies
  Organized by Mizoram University and NRDMS-DST, Govt. of India, New Delhi.

- International Conference on Recent Advances in Animal Sciences (ICRAAS) 2019.
  Organised by- Department of Zoology, Pachhunga University College, Aizawl , Mizoram.

ABSTRACT

DIVERSE EFFECTS OF DEGREE OF URBANIZATION AND FOREST SIZE ON BEETLES BIODIVERSITY AND SPECIES COMPOSITION IN AIZAWL, MIZORAM

MALSAWMDAWNGZUALI TARA

DEPARTMENT OF ZOOLOGY
MIZORAM UNIVERSITY

INTRODUCTION

Beetles belong to the order Coleoptera, the largest order of insects and has an estimated 3,50,000 species (www.zin.ru/Animalia/Coleoptera) that are classified into 160 families (Elzinga, 1992). These insects, which are characterized by their thick and tough forewings, or elytra, have adapted to various habitats from aquatic to soil, to trees, foliage, and other aerial surfaces. They can range in size from 1 mm to approximately 75 mm in length (Borror *et al*., 1976). In India alone, more than 15,500 species of beetles have been recorded (Sengupta and Pal, 1998).

Beetles play a major role in the forest ecosystem and are very sensitive to ecological disturbances (Filgueiras *et al.,* 2011). Some of the beetle families which are found to be predominant in tropical forests have been employed as bioindicator species in biodiversity monitoring programs to assess forest cutting, alteration in landscape, habitat degradation, fragmentation-related effects, organization patterns change in species composition, farming intensification, and anthropogenic environmental disturbances (Audino *et al.,* 2014; Barnes *et al.,* 2014; Campos and Hernández 2015; Filgueiras *et al.,* 2011, 2015; Korasaki *et al.,* 2013).

Beetles have also been known to be used in forest ecosystems where their species diversity and/or abundances change along a habitat disturbance gradient; and is found that while specialist species may decrease with increase in disturbance, the generalist species with good dispersal ability are increasing. At the same time, some species may not be affected by restrained disturbance.

In recent years, there has been a number of works which have been carried out on many Coleopteran families like, Staphylinidae, Scarabaeidae,Carabidae, Cicindelidae and Psephenidae to designate and use them as ecological indicators for many purposes apart from other orders of class Insecta like Trichoptera , Ephemeroptera and Plecoptera. The presence and condition of beetles also give a more accurate information about the health of the ecosystem as their behaviour is directly related to the anthropogenic-induced landscape modification in the form of agricultural fields, plantations, and urbanisation. (Bhargava, 2009)

Urbanization refers to the population shift from rural areas to urban areas. It is the gradual increase in the proportion of people living in urban areas, and the ways in which each society adapts to this change. Urbanization is increasing globally and its related factors which include increase in spatial isolation and decrease in the habitat size can change the dynamics of plant and animal populations in urban green areas (Niemela, 1999; McKinney, 2002). There have been several studies along urbanization gradients which reported alterations in abiotic conditions in the remaining habitat patches caused by changes in temperature, precipitation and nitrogen deposition from the rural surroundings to the city centre (Bai *et al.,* 2008; Gilbert, 1994; Irwin *et al.,* 2011). All these changes can influence habitat quality and, subsequently, the species richness, species composition and functional diversity of plants and animals (McKinney, 2002; Sukopp, 1998; ConcepcioÂn, 2015), which in turn affect the functioning of ecosystems (Sala *et al.,* 1997).

Anthropogenic activities that causes changes to the forest and landscape which pertains to towns and cities causes major changes to the ecosystem. Differences in a number of variables such as temperature (Bornstein, 1968), acidity, soil hydrophobicity, utilizable carbon and nitrogen levels (McDonnell *et al*., 1997), deposition of heavy metals (Hynninen, 1986) and other pollutants (Herrmann and Hübner, 1984; Väisänen, 1986), changes in fungal biomass, bacterial flora, leaf-litter decomposition rates (McDonnell *et al*., 1997), fragmentation and edge effect (Löfström *et al*., 1999), all contribute to the effect of urbanization. In addition, the highly significant physical covering of potential habitat with asphalt and cement causes direct loss of habitat.

The identification of the main factors that drive the composition of communities and the distribution of species is an essential objective in community ecology and is of particular importance for envisaging biodiversity responses to environmental changes (Belyea and Lancaster, 1999). Ozinga *et al*. (2009) showed that differences between plant species in characteristics involved in dispersal processes contribute significantly to explaining losses in plant diversity in response to habitat degradation.

Due to their extreme species richness, beetles, coupled with the morphological, as well as ecological and behavioural diversity, and are being widely used for environmental impact assessments, ecological studies and monitoring activities (Bicknell *et al*., 2014). While their significance for environmental impact assessment may be undisputed, there still remains a need to better understand the habitat requirements, especially of their larvae, as well as interactions between species (Rainio and Niemela, 2003). This often requires superior taxonomic tools. This is where DNA barcoding come into play as it can provide an efficient method for biodiversity assessment as it meets the need for a faster, more efficient and reliable species identification at this time of climate change and massive habitat destruction (Valentini *et al*., 2009). This approach may possibly be a much better way for handling the vast diversity of invertebrates which are critical for ecosystem functioning while at the same time often poorly known taxonomically. DNA barcoding also has the power to connect different life stages of the insects. As such, it can link hundreds of years of taxonomic, ecological, faunistic and ethological studies with ultra-high-throughput sequencing of the genomic age. The latter approach will have great benefits for many application-oriented fields, especially in agriculture and forestry where the swift and reliable identification of bulk samples is often required.

REVIEW OF LITERATURE

An increase or a decrease of beetle species number or abundance might be directly caused by change in environmental factors or indirectly by change of species assemblage of other species (Rainio and Niemela, 2003).

Several urban studies reported a replacement of forest specialists by generalist species with increasing degree of urbanization suggesting that forest specialists are more sensitive to urbanization- related disturbances (Deichsel, 2006; Vergnes, 2014; Magura, 2010).

Some of the beetle families which are found to be prevalent in tropical forests include Scarabaeidae, Carabidae, Bruchidae, Buprestidae, Cantharidae, Cerambycidae, Chrysomelidae, Coccinellidae, Curculionidae, Elateridae, Lampyridae, Staphylinidae, Melolonthidae, Lucaenidae, and Tenebrionidae. They have been employed as bioindicator species in biodiversity monitoring programs to assess forest cutting, alteration in landscape, habitat degradation, fragmentation-related effects, organization patterns change in species composition, farming intensification, and anthropogenic environmental disturbances (Audino *et al.,*, 2014; Barnes *et al.,* 2014; Campos and Hernández 2015; Filgueiras *et al.,* 2011, 2015; Korasaki *et al.,* 2013).

The first report on the family cerambycidae from the state of Chhattisgarh accounts for 10 species of Cerambycid beetles belonging to eight genera and six tribes under two subfamilies (Majumder, 2014).

There are a number of new locality records for beetle species, including first state records for Mizoram, of 92 species of *Sericini* (Coleoptera: Scarabaeidae: Melolonthinae) from the Indian subcontinent. Eight new species are described : *Maladera alloservitrita* , *M. kolasibensis*, *M. mizoramensis*, *Neoserica radhanagariensis, Serica basantapurensis, S. mahakaliensis, S. therathumensis,* and *S. zianii* . (Shreedevi *et.al.,* 2018).

The number of cerambycid species recorded from India is about 1500 (Beeson 1939; Breuning, 1966) including 13 species reported from Tripura (Mukhopadhyay and Biswas, 2002). The pioneering taxonomic and biological investigations on cerambycid beetles in India were initiated in the 20th century. Gahan (1906) was the first to compile and describe the known cerambycid beetles, excluding Lamiinae, from the Indian region in the 'Fauna of British India'. After that, extensive work on the diversity and distribution of cerambycids from India is particularly lacking (Majumder et al.,, 2014).

Forty nine species of cerambycids belonging to three subfamilies were recorded from Arunachal Pradesh, India. Subfamily Lamiinae was found to be dominant with 28 species followed by Cerambycinae with 11 species. Subfamily

Prioninae included 10 species. *Rhytidodera griseofasciata* reported from China earlier is being reported from India for the first time during the study (Kumawat 2015).

It has been surveyed that 9,00,000 species of insects are described globally in which 6,00,000 species were located and described in India, where many of them are yet to be described or named (Balaji,2016).

Plant species richness, the percentage of forest specialists and Shannon diversity of plants were affected by the degree of urbanization. While the species richness and Shannon diversity of plants decreased with increasing degree of urbanization, the percentage of forest specialists was slightly higher in forests located in areas with either a low or high degree of urbanization compared to forests situated in areas with a medium degree of urbanization. Furthermore, Shannon evenness of plants tended to decrease in forests with increasing percentage cover of sealed areas in their surroundings. (Mellinger, 2018).

Several urban studies reported a replacement of forest specialists by generalist species with increasing degree of urbanization suggesting that forest specialists are more sensitive to urbanization- related disturbances (Deichsel, 2006; Vergnes, 2014; Magura, 2010). This finding was unexpected and may be a result of combined effects of differences in habitat diversity in the surroundings, which may be highest at medium levels of urbanization, and of refugia effects of forests in highly urbanised areas (Mellinger, 2018).

Not all species respond to environmental changes caused by urbanization in the same way, because they have different requirements regarding their habitat and its surrounding landscape (ConcepcioÂn, 2015; Godefroid, 2007; McIntyre, 2001). For example, specialist species may perceive the surrounding matrix as a stronger barrier than generalists, which are able to exploit a wide variety of resources from neighbouring green areas (Bai X *et al*., 2008; Croci, 2008). Thus, specialist species become frequently replaced by generalists (Gibb, 2002; Magura, 2004). As a result,

species composition in urban areas becomes more and more similar, which in turn may lead to a decrease in functional diversity homogenisation (LizeÂe, 2011). Furthermore, groups of species at high trophic ranks such as herbivores and predators might also be more influenced by increased isolation and habitat loss because of their dependence on other species compared to groups of species at low trophic ranks such as plants (Holt, 1999; Steffan-Dewenter, 2003).

Rapid identification of unknown specimens may be achieved through DNA barcoding and query of public sequence databases (Collins and Cruickshank, 2012). This approach has been adopted for use in regulatory fields such as conservation biology, consumer protection, and border biosecurity, especially when morphological identification of target taxa is difficult and/or impossible (Collins *et al*., 2012).

The ability of DNA barcoding to link immature life stages to adult beetles makes it a valuable tool for overcoming the challenge presented by conserved larval morphology (Hendrich *et al*., 2015). However, the application of DNA barcoding depends on the availability of matching reference barcodes within public databases, where the order Coleoptera is still underrepresented compared with Hymenoptera, Diptera, and Lepidoptera (Boykin 2015; Woodcock *et al*., 2013).

OBJECTIVES

The objectives of the present study are as follows:

1. Cataloguing and biodiversity analysis of beetles in Mizoram in relation to the effect of urbanization and forest size.

2. To establish a barcoding system and assess the species composition in relation to urbanization and forest size.

MATERIALS AND METHODS

4.1. Ethics statement

All necessary permits will be obtained from the Chief Conservator of Forests, Department of Environment and Forests, Ministry of Environment and Forests, Mizoram for the field studies. (Permit Number: No.B. 11015/19/2007-FST).

4.2. Study Area

The study was carried out in Aizawl (23.7307° N, 92.7173° E), Mizoram. The study area covers 457 sq. km and located at 1132 m a.s.l. It is located north of the Tropic of Cancer and have a mild to humid sub-tropical climate due to its location and elevation.

Inside the study area, 5 sites were taken into consideration to compare the degree of urbanization between the different areas.These sites were chosen along the urbanization gradient from the urban,i.e, the area lying inside the city, forest or rural area and the semi-urban area which is in between the two.

The study was conducted between October 2018 to September 2019 and sampling was carried out on a bi-monthly basis at the different study sites which are:

A) Mission veng (23˚ 42’50.56” N, 92 ˚43’05.86”E)
B) Kanan (23˚ 42’02.90” N, 92 ˚43’35.09”E)
C) Sakawrtuichhun (23˚ 45’40.07” N, 92 ˚40’15.81”E)
D) MZU, Tanhril (23˚ 44’26.27” N, 92 ˚39’23.34”E)
E) PUC, College veng (23˚ 42’23.73” N, 92 ˚43’37.99”E)

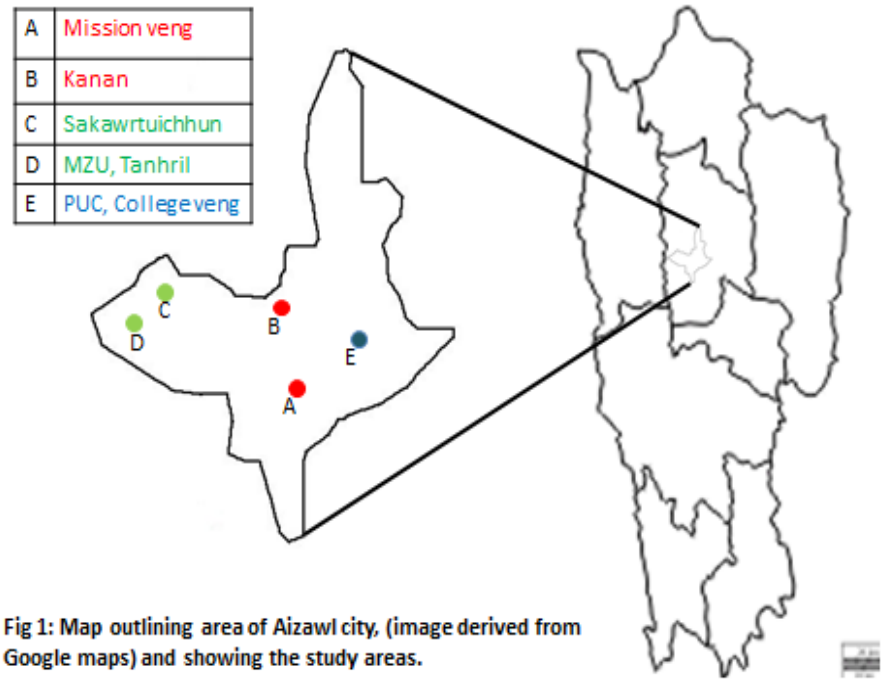

Fig 1: Map outlining area of Aizawl city, (image derived from Google maps) and showing the study areas.

Sampling of Beetles:

For the sampling of beetles, the methodology as described by White (1998) was primarily followed. Other methods were also used as convenient.

Light trap method was the most dominant method employed during the study for collection of nocturnal species, where lights were placed in the centre of the fields at a height of about 3 meter above the ground and operated between 9:00 PM to 12:00 PM to attract the beetles.

For beetles which are not active at night, manual collection was also carried out by going to the study sites and checking the foliages and trees. Hand picking from the leaves and flowers as well as shaking and beating of the bushes and tree branches to disturb the beetles which were then captured manually or sometimes by using nets for sweeping ground beetles.

The trapped beetles were collected and separated family-wise and the cumulative count was determined at each location. The specimens collected were then stored in 70% alcohol until further use. (Ibrahim *et al*., 2016)

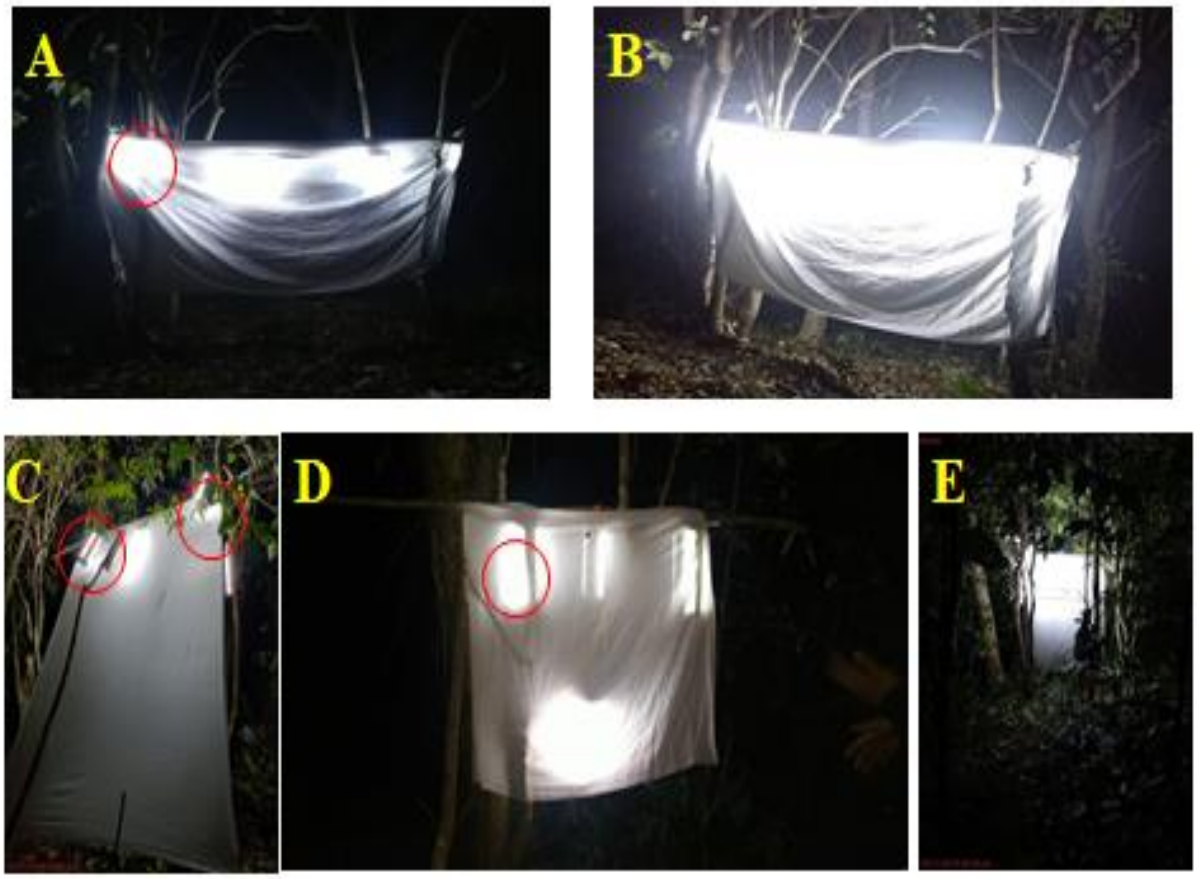

Fig 2: Light traps placed at different study sites (A) Mission veng (B) Kanan veng (C) Sakawrtuichhun (D) MZU campus, Tanhril (E) PUC campus, College veng

Morphological Identification of Beetles

The identification will be done up to family level using identification keys and by studying the morphological structures of each specimen (White,1998; Castner, 2000).

For the present study, the morphological features like the size and shape of antenna and legs, the shape and colour of the body, etc., were examined and recorded for use in the process of identification. The specimens collected from the study sites were thoroughly examined in the laboratory in order to confirm the initial result and to record the numbers belonging to each family using the identification keys.

Landscape characteristics and recreational pressure

The urbanization gradient of the sampling sites was determined by the percentage cover of built-up area and traffic infrastructure, urban green space, agricultural land and forest cover, and the percentage cover of sealed area was determined to use as a measure of the degree of urbanisation. (Melliger *et al.*,2018*)*

Land cover data of the landscape characteristics from satellite images (Google Earth, 2009) was derived. Around the most central sampling plot in each area, the percentage cover of built-up area and traffic infrastructure, urban green space, agricultural land and forest cover within radii of 200 m and 500 m was determined using the pixel counting function of Adobe Photoshop (version 10.0.1). (Melliger *et al.*,2018*)*

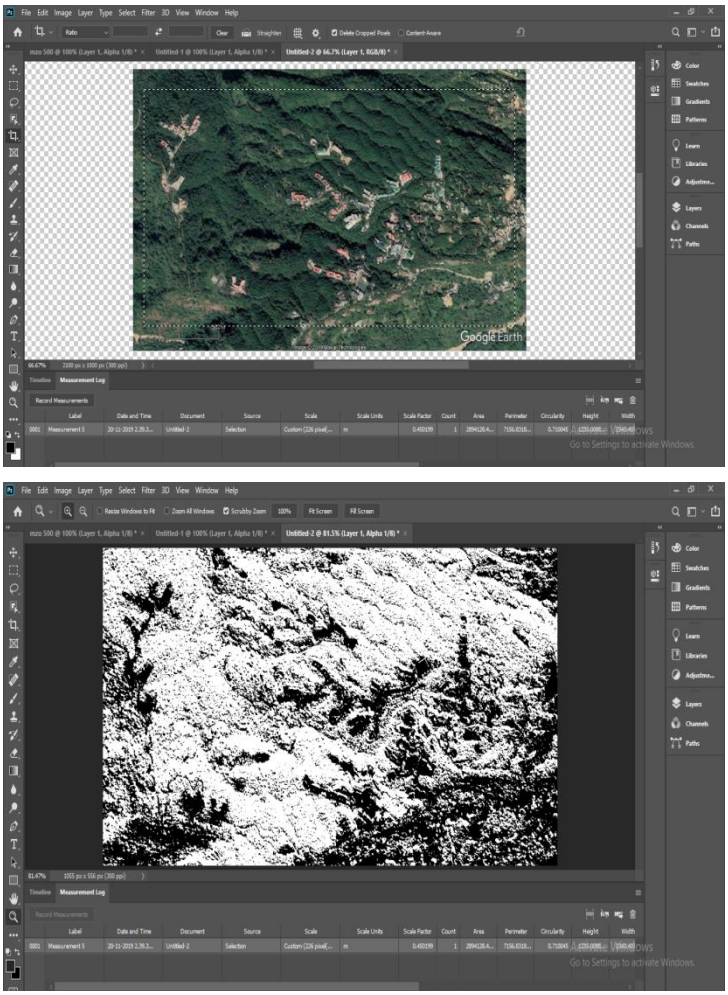

Fig 3: Calculation of percentage cover from Google Earth image using Pixel counting function of Adobe Photoshop

Diversity Data Analyses:

The following indices were computed:

1. Species diversity indices (i.e., Shannon–Weiner index and Simpson index),
2. Species richness indices (i.e., Margalef index, DM =[(S-1)/ Ln(N)] and Menhinick index),
3. Species evenness and dominance indices (equitability J index or Pielou's evenness index [J'= H'/log S] and Berger–Parker index [d =Nmax /NT]), and where pi= ni/N, ni is the number of individuals of species, i and N are the total number of individuals, S is the number of species, Nmax is total dominant species in a habitat type, and NT is the proportion of the total species (Ibrahim, 2016).

4. Principal component analysis (PCA) was also performed using PAST (version 1.86b) software (Hammer et al., 2001) to investigate and ordinate the relationship between the beetle families' composition and their community association with the habitat. Thus, the axes derived correspond to gradients of species compositional change.

Genomic DNA extraction

The extraction protocol by Sambrook et al. (1989) was followed. For the DNA extraction, the legs were washed with double distilled water and dried. The legs were macerated with the help of scissors in 1.5 mL Eppendorf tube, and homogenized with pestle, and 250 µL of extraction buffer (100 mM Tris HCl, 200 mM NaCl, 50 mM EDTA, 1 % SDS) was added and mixed gently. Proteinase K (20 mg/mL, 2 µL) was then added followed by incubation in an oven at 56 °C for 30 min. To this, 250 µL of phenol/chloroform (1:1) was added and mixed gently and centrifuged at 13,000 rpm for 5 min. Supernatant was then carefully taken out and collected in a new Eppendorf tube. Absolute ice cold ethanol (450 µL) was added to the supernatant and mixed gently by inverting the tube several times and kept in -20 °C for 30 min. The tube was next centrifuged at 13,000 rpm for 5 min at 4 °C. Ethanol was poured off

without dislodging the pellet and 200 µL of 70 % ethanol was added and flash spun at 6000 rpm for 1 min. The ethanol was poured off and the pellet dried. Double distilled water (30 µL) was then added to the tube; and the pellet resuspended by gently flicking the tube and later stored at -20 °C for further use.

Genome sequencing

The mitochondrial cytochrome c oxidase I (COI) region, which is used in most DNA barcoding analyses of insects, exhibits substantial variation in every one of three nucleotides (i.e., the third codons), even among species in the same insect orders, introducing taxonomic bias into prey community data. PCR was performed with two universal primers; COI gene: forward primer LepF1 (5′-ATTCAACCAATCATAAAGATATTGG-3′) and reverse primer LepR1 (5′-TAAACTTCTGGATGTCCAAAAAATCA-3′) (Hebert *et al*., 2004). The 25-µL reaction mixture contained 1X amplification buffer, 2.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.2 pM each forward and reverse primer, 0.8 µL BSA, 2 µL genomic DNA, and 1 U Taq DNA polymerase. The PCR thermal regime for amplification used was at 5 min at 95 °C for initial denaturation, followed by 30 cycles of 30 s at 95 °C for denaturation, 40 s for annealing at 51-54 °C, elongation for 30 s at 72 °C, and a final elongation for6 min at 72 °C. PCR products were checked by gel electrophoresis on a 1.5 % agarose gel containing ethidium bromide. Successfully amplified DNA fragments were purified. Samples were then sequenced using Sanger's di-deoxy method, and sequencing reactions were carried out in both directions on a sequencer. All the sequences were checked using BLAST (NCBI).

Analysis of COI sequences using MEGA 6.0and phylogenetic inference

The sequences of COI from the beetles were aligned using MUSCLE (Edgar, 2004) implemented in the program MEGA 6.0 (default settings retained except maximum number of iterations (maxiters= 1000). Identical sequences were removed. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 6.0 (Tamura *et al*., 2013). Model test (best-fit substitution model) and

substitution parameters (test pattern homogeneity, substitution pattern, rate variation among sites, transition/transversion bias, disparity index, amino acid composition, nucleotide composition, codon usage bias, and site-by-site rates) were estimated using maximum composite likelihood method. Pairwise distances (in over all-, within-, and between-group and net between group mean distances), diversity (in entire population, mean inter-population, and coefficient of differentiation), bootstrap, and analytical variances were also computed. Computed distances were separated based on site degeneracy, codon sites, transitions and transversion bias, and non-synonymous and synonymous changes. Codon-based Z test and Fisher's exact test of selection and Tajima's test of neutrality were performed. Gamma parameter for site rates, position by position rates, and Tajima's relative rate test were done. The analysis involved 11 nucleotide sequences of COI gene from beetles. Codon positions included were first + second + third+ non coding. All positions containing gaps and missing data were eliminated (Tamura *et al*., 2013). The ratio of the number of non-synonymous nucleotide substitutions per site (dN) to that of synonymous nucleotide substitutions (dS) based on a set of aligned COI sequences were performed to investigate the selection pressure among the beetles COI gene by using online tool SNAP v2.1.1 (Korber, 2000). Phylogenetic relationships were inferred using Maximum Parsimony (MP) for COI datasets. MP trees were obtained using PAUP 4.0b10 (Swofford, 2002) by heuristic search option with tree-bisection-reconnection (TBR) branch-swapping. The number of bootstrap replicates was set at 1000.

Sequence statistics and identification success

BOLD tool was used to calculate the nucleotide composition of the sequences and distributions of Kimura-2-Parameter distances within and between species. The performance of barcode sequences in species identification was assessed by conducting a barcode gap analysis in BOLD. All species found to share haplotypes with one or more other species were interpreted as identification failures.

Barcode Index Numbers

The Barcode Index Number (BIN) system were created as an interim taxonomic system to aid management of the 3 M barcode sequences in BOLD. Sequences were assigned to BINs using the Refined Single Linkage (RESL) algorithm which performed an initial single linkage analysis employing 2.2% sequence divergence as a minimum distance between clusters. The resulting operational taxonomic unit (OTU) boundaries were then refined by Markov clustering. The BIN assignments on BOLD are constantly update as new sequences were added, and individual BINs were split or merged in light of new data. The BIN assignments used in this study were downloaded from BOLD. Ratnasingham and Hebert (2013) scheme was used for comparison to examine the correspondence between traditionally recognized species and the OTUs delimited by the RESL algorithm. Each species was assigned to one of four categories as follows: (1) Match: all specimens of a species included in one BIN; (2) Split: specimens of a single species divided into two or more BINs; (3) Merge: all specimens of two or more species combined into a single BIN; (4) Mixture: Both a merge and a split involving two or more species.

Soil analysis:

Top soil samples (0–10 cm) were randomly collected from each sampling site, and stored in plastic bags. The composite soil samples were used for soil analyses. Soil pH, organic carbon, available phosphorus, sulphur and total nitrogen were measured following the standard protocols (Anderson and Ingram 1993; Black *et al.,* 1965). Evaluation to assess the correlation between soil health and diversity of beetle families was also done using PAST 3.25 Software.

Vegetation analysis

The analysis of vegetation was applied based on line transect method of 50 x 50 m^2 of five quadrates analyzed (5x50m = 250m^2) with five sub-plots each at four corners (5x10 = 50 m^2). All trees >20 cm in diameter at breast height (DBH) shrub, climber, vines and lianas >5 cm were measured and identified within each plot. Apart

from tree density, the vegetation was also analyzed for relative frequency, relative density, relative dominance and species IVI (Mishra, 1989; IJSC, 2015).

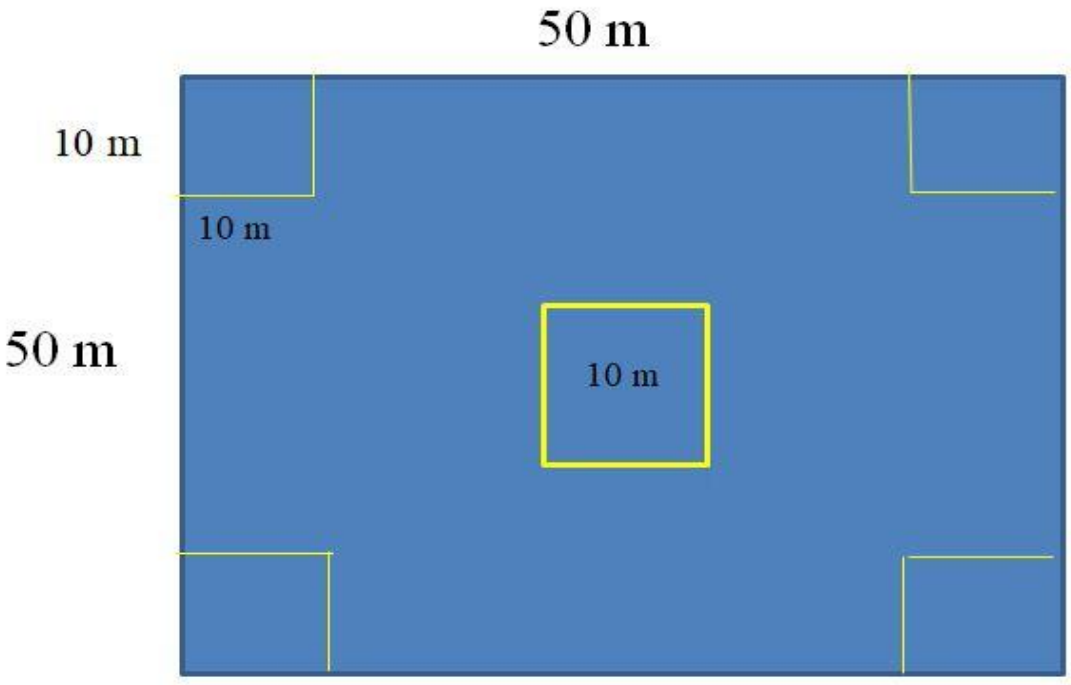

Fig 4: Quadrat with sub-plot

Summary of results:

1. The study was conducted from October 2018 to September 2019, for a period of 12 months in 5 sites in and around Aizawl city which are represented as rural or forest, semi-urban and urban sites as the study pertains to the effect of urbanization on beetles diversity. During the study period, a total 445 beetles belonging to 15 families were collected. The study showed that the beetles diversity was highest in forest site, followed by urban site while it is moderate to less in the semi-urban site.
2. Out of the 15 families recorded, the total number was recorded from the Cerambycidae family which could be found on all the 5 different sites followed by Lucanidae and Scarabidae. The family Buprestidae,

Lampyridae and Meloidae were regarded as habitat specialists as they were found only is certain habitats. There are also some specific species from other families which are highly specialized in their habitat while some others from the same family were not found to be so as in the case of Chrysomelidae and Curculionidae.

3. It was also observed that the collection of beetles was most successful during the spring and summer seasons as compared to the winter season while the rainy season sampling results in only few selective species and the more common generalists.
4. Statistical analyses were also performed which showed the most diversity and dominance in the forest or rural sites, while richness is more in urban sites. Further, after correlating between the plant-soil-beetle diversity, the results showed that the soil parameters are directly affecting the occurrence of the plants in an area and this in turn affect the beetles biodiversity and species composition. This can also be stated in another way which goes as the degree of urbanization increases in an area, the diversity of beetles will decrease, which directly is in line with our hypothesis.
5. The second part of the study which is the barcoding of DNA of beetles was also performed satisfactorily. A total of 76 beetles belonging to the 15 families were taken DNA extraction and sequencing. The sequences obtained were given BIN number and then submitted to NCBI database, and at the same time, phylogenetic analyses were also conducted to determine the relationship and distance between the different beetles species and families as a whole.

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