CHAPTER –2

ECOLOGY OF MYRMELEONTID LARVAE

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2.1. INTRODUCTION

Ecology is the study of interactions among organisms and between organisms and their environment. The interactions can be biotic and abiotic; biotic interactions are interactions among living organisms and those between organisms and their physical environment are called abiotic interactions. The four main areas of ecology are Organismal, Population, Community and Ecosystems ecology. The organismal ecology divided into three main subdivisions; that is evolutionary ecology, behavioural ecology and physiological ecology. Here the behavioural ecology of antlions was explained in two separate sections as ecology and behaviour. Behavioural ecology explains how the behaviour of an organism contributes to its survival and reproductive success which inturn affects the abundance of a population (Stiling, 2012). Antlion larvae have some strategy to increase its survival rate in its environment in which seasonal adaptability, high fecundity rates are some of the examples.

The study of ecological aspects of an individual or a species denotes the habit and habitat study in detail. In the case of ecology study of antlions, the different species present in a particular area (Kerala), the number of species present in one habitat, morphological peculiarities, physical parameters of habitat, natural enemy, seasonal adaptability, habitat choice are to be mentioned. Though the antlion larvae are terrestrial in nature, the organizing component of terrestrial ecosystems has more importance. For the study of abiotic factors and organism a new branch is present called ecophysiology. Ecophysiology is the part of ecology deals with the response of individual organisms or species to abiotic factors such as temperature, light, moisture, atmospheric gases and other factors in the environment (Barrick *et al*., 2005).

According to the latest classification work done in India (Ghosh, 2000) and World antlion Catalogue (Stange, 2004) a comparison of presence of antlions were made for understanding the status of antlion species both in India and World.

Stange-2004 (World Antlion)

Family Myrmeleontidae

1. Subfamily Stilbopteryginae

2. Subfamily Palparinae

Tribe Palparidini , Tribe Palparini , Tribe Pseudimarini , Tribe Dimarini

3. Subfamily Myrmeleontinae

Tribe Maulini, Tribe Dendroleontini, Tribe Nemoleontini, Tribe Brachynemurini Tribe Gnopholeontini, Tribe Lemoleontini, Tribe Myrmecaelurini, Tribe Nesoleontini, Tribe Myrmeleontini, Tribe Acanthaclisini

4. Subfamily Araripeneurinae

5. Subfamily Paleoleontinae

6. Subfamily Unknown

Ghosh-2000 (North East India)

Family Myrmeleontidae (Table 1 and Table 2)

1. Subfamily Palaparinae

Tribe Palparini (1 genus)

2. Subfamily Myrmeleontinae

Tribe Dendroleontini (4 Genus), Tribe Acanthaclisini (2 Genus), Tribe Myrmeleontini (3 genus), Tribe Distoleontini (5 Genus), Tribe Glenurini (1Genus)

Table 1. Subfamily Myrmeleontinae- Species reported from India

Sl No	Tribe	Genus	Species
1	Dendroleontini	*Layahima*	*L. nebulosa Navas*
		Indoclystus	*I. singularis (Westwood)*
		Dendroleon	*D. regius (Navas)*
		Gatzara	*G.jubilaea Navas*
2	Acanthaclisini	*Centroclisis*	*C. horridus (Walker)*

		Stiphroneura	*S. inclusa (Walker)*
3	Myrmeleontini	*Myrmeleon*	*M. clothildeBanks,* *M. montanus Navas,* *M. assamensis Ghosh,* *M. berenice Banks*
		Hagenomyia	*H. sagax (Walker),* *H. marginicollis(Gerstaecker),* *H. eurystictus (Gerstaecker),* *H. nigrinus (Esben-Petersen),* *H. monticolla (Navas),* *H. jamduarensis Ghosh*
		Talosus	*T. oberthurai Navas*
4	Distoleontini	*Creoleon*	*C. griseus (Klug)*
		Allogama	*A.irene (Banks)*
		Neuroleon	*Neuroleon sp.*
		Distoleon	*D. verendus (Walker),* *D. bivittatum Banks,* *D. sambalpurensis Ghosh,* *D. audax (Walker)*
		Dolicholeon	*D. substigmalis Navas*
5	Glenurini	*Negrokus*	*N. lebasi Navas*
	Uncertain position	*Baga*	*B. montana Navas*

Table 2. Subfamily Palparinae- Species reported from India

Sl No	Tribe	Genus	Species
1	Palparini	*Palpares* Rambur	*P. pardus Rambur* *P. contrarius (Walker)*

Though the larval antlions are present in soil, the development of larvae into adult was highly influenced by the soil texture and composition of that particular area. Soils of different ecosystems or climate have special properties such as different

colours and compositions (Colinvaux, 1986). Soil formation is influenced by the climate, vegetation and hydrological conditions of the particular area and according to this, lots of soil classifications are present. Here, the classification of soil types were taken from Kerala agriculture website (http://www.keralaagriculture.gov.in) and the comparison of soil type and antlion larval presences were studied (Table 3).

Table 3. The six type of soils in Kerala

Sl No	Soil Type	Description
1	Coastal Alluvium	80% sand content and up to 15% clay content , The water holding capacity is poor
2	Alluvial Soil	Kuttanad and Kole lands of Thrissur district. Sandy clay loam to clay
3	Kari soil	Chittur/Palakkad. Clay loam to clay
4	Laterite soil	p^H-5.0-6.2
5	Red soil	Thiruvananthapuram. Sandy clay loam to clay loam. With red to dark red colour
6	Hill soil	Loam to clay loam

The soil is composed of sand, silt and clay particles. According to the International Union of Soil Sciences (IUSS), the soil particles were classified as follows (Table 4).

Table 4. Soil particle classification by IUSS

Particle	Diameter (mm)
Coarse sand	2.0-0.2
Fine sand	0.2-0.02
Silt	0.02-0.002
Clay	<0.002

Like habitat, another important aspect of ecological study rely on how an organism get its food and how they were food by another organism. The former is called

feeding habit and the latter termed as natural enemy. The ecology study of an organism will complete only by mentioning these areas in a proper way. In the case of antlions, the major importance is given to the larval forms because of the presence of specific feeding habits, natural enemies, specific habitat etc.

2.2. REVIEW OF LITERATURE

2.2.1. Myrmeleontids of World

Numerous studies were done by using different species of pit building antlions, but in the case of diversity or quantifying different species, the previous works were less when compared to behaviour study. Iran, Malesia, China, United Arab Emirates, Pakistan are some countries in which quantification of antlion species were done efficiently. New (1982) described Newzealand species of antlion, *Weeleus acutus* with structure of wings, genitalia and its larval stage. In the case of India, after 1984 not much work was conducted and from Southern part of India unfortunately not a single species was reported. New and Sudarman (1988) described the Neuropterans of Krakatau Islands, Indonesia in which *Myrmeleon frontalis* was the only species present there, it is one of the earlier study and report of a species. Poinar and Stange (1996) gave knowledge about fossil records of Myrmeleontids and also described a new Dominican Amber antlion species that is *Porrerus dominicanus*.

In 2002, Mirmoayedi presented a list of 23 species of Neuroptera from Iran, in which 9 species are coming under Family Myrmeleontidae. Stange (2004) consolidated the data regarding the world antlion species and the works of all eminent scientists in the field of antlion research were included. In this book he explained about 1522 extant species, 13 fossil species, 201 genera, 14 tribes and 5 subfamilies including 2 fossil subfamilies. The Subfamilies are Stilbopteryginae, Palparinae, Myrmeleontinae and the remaining Araripeneurinae and Paleoleontinae are fossil subfamilies. It is considered as the latest consolidation of family Myrmeleontidae, with valid taxonomic keys, distribution, parasites, predators, which includes the works from the year 1700 to 2000.

With a gap of 4 years Mirmoayedi (2006) again contributed to antlion fauna of Iran with 7 new records. In the same year Bao and Wang reviewed the species of *Euroleon* from China. Instead of Mirmoayedi, Abraham (2007) also presented a new species of *Macronemurus* from Iran. In the year of 2008, Saji and Whittington recorded 27 species of antlion from Abudhabi Emirate followed by Dong and Engel, they described a new fossil antlion from North Eastern China.

Scudder and Cannings (2009) made a checklist of Neuroptera of British Columbia, in which 5 species were coming under Family Myrmeleontidae. It includes 3 species of genus *Brachynemurus*, *Dendroleon* and *Myrmeleon*. Abraham (2010) described a new *Palpares* species from Middle East Asia and Devetak *et al*., (2010a) studied the morphology of non pit building antlion larvae *Neuroleon microstenus* in the same year. Devetak *et al*., again contributed about the morphology of *Myrmeleon yemenicus* Holzel in the same year (2010b). Pantaleoni *et al*., (2010) described a new Mediterranean species *Myrmeleon mariaemathildae* from Sardinia and Tunisia.

The next decade starts with the work of Miller and Stange (2011), and they published a paper about the antlions of Hispaniola; Abraham and Dobosz listed 27 antlion and 7 owl fly species from Madagascar in this year. Michel and Akoudjin (2012) reviewed the *Neuroleon Navas* of West Africa with 4 new species descriptions. Zhan *et al.*, (2012) presented a synopsis of the genus *Deutoleon Navas.* Pantaleoni and Badano reported a new species of pit building antlion *Myrmeleon punicanus* from Sicily and Pantellaria (2012). In the same year Zhan and Wang (2012) described a new species of *Bankisus Navas* (*Bankisus sparsus sp*) and also provided a Key to Bankisus. A huge work was come from Miller and Stange (2012) about cave mouth antlions of Australia in which they described 12 new species.

Devetak *et al.*, (2013) studied about the antlions of Albania with 14 species and Acevedo *et al.*, (2013) described the larvae of European species *Distoleon Banks* in the sam year. Krivokhatsky *et al*., (2014) got three species of antlion from Curonian spit Russia from bird traps and the species includes- *Myrmeleon tschernovi*, *M. Formicarius* and *Euroleon nostras* in the ratio 100:3:2. In the same year four rare species of antlion from middle Asian countries were reported by Khabiev and Krivokhatsky (2014). Badano and Pantaleoni (2014) published a most important work which is helpful for many emerging scientists in the field of Myrmeleontids that is larvae of European Myrmeleontids with taxonomic key. Acevedo *et al.*, (2014) in the same year described the larvae of *Tricholeon relictus* Holzel. Michel (2014) revised the Genus *Solter* Navas 1912 of Maghreb and West Africa with descriptions of 5 new species. Myrmoayedi *et al*., in (2015) made a check list of antlions of Iran.

Devetak (2016) contributed a checklist of Lacewings of Albania and Abraham (2017) described *Myrmeleon* species from Sichuan, China. Krivokhatsky *et al.*, (2017) in the same year described *Palpares turcicus* in the Iranian fauna. Akhtar *et al*., (2018) reported 5 species of Genus *Myrmeleon* from Pakistan and Badano *et al.*, reviewed the antlions of Cyprus with 7 new reports. Hamouly *et al.*, (2019) reviewed subfamily Palparinae of Egypt and Krivokhatsky (2019) noted two size ranges of *Myrmeleon hyalinus hyalinus* from UAE. The larval morphology of 3 Afrotropical pit building antlion genus *Myrmeleon* by Badano published in 2020 is considered as the latest work published regarding antlions.

2.2.2. Myrmeleontids of India

An earlier knowledge about Neuroptera was found in ZSI, in which a book named 'Animal resource of India' (1991) has found as a historical resume of Neuropterida and estimated 37 genera of Myrmeleontidae and 125 species. The Records of the ZSI gave knowledge about Neuropteran species of Himachal Pradesh. A total of six species were described which includes *Palpares pardus* (Tribe *Palparini*), Tribe *Distoleontini*, Tribe *Acanthaclisini* and Tribe *Myrmeleontini*. Ghosh and Sen in 1977 published a checklist of Indian Planipennia (Order Neuroptera) and Ghosh (1983) reported Neuropteran from North-West Himalayan and Northern peninsular sectors of India. Ghosh again reported Neuroptera from Rajasthan (1977), Orissa (1987) and Lakshadweep Islands (1990).

Alfred *et al*., in 1998 wrote a book named 'Faunal diversity of India' depict that 335 species, 125 genera and 13 families of Neuropterans are recorded from India in which 40 genera and 126 species are coming under family Myrmeleontidae. In the same year Ghosh again contributed about collection, and preservation of Neuroptera, external morphology and terminology and systematic accounts. Here, Key to the genera of *Palpares, Centroclisis, Cueta, Nesoleon, Hagenomyia, Myrmeleon, Creoleon, Gatzara, Neuroleon* and *Distoleon* were presented. A consolidated list of 73 species of neuropteran from West Bengal was recorded in this paper.

Ghosh (2000) consolidated the Neuroptera of North-east India (Arunachal Pradesh, Assam, Meghalaya, Manipur, Nagaland, Mizoram, Tripura, West Bengal, and Sikkim) and 128 species in 69 genera and 11 families were reported. In the book named 'Insects of India', Sengupta (2005) found that among the 100 species of antlion recorded from India, 45 species was from North East India and approximately 12 families and 35 species of Neuropterids were recorded from India. Sharma and Chandra (2012) recorded 60 species of Neuropterans from Maharashtra in which family Myrmeleontidae consists of two subfamilies (Palparinae and Myrmeleontinae) and 5 tribes and a checklist was made. Three species of antlion were reported from Chattisgarh such as *Siphoneurainclusa* coming under Subfamily Myrmeleontinae and *Stanaresimprobus*, *palparespardus* coming under Subfamily Palparinae with detailed description (Chandra *et al*., 2014). Kaur *et al.*, (2019) studied about the female and male genitalia of *Myrmeacaelurus acerbus*.

2.2.3. Abiotic factors

Abiotic factors like temperature and humidity have some effects on every organism, sometimes the abiotic factors influence the lifecycle and behaviour also. Arnett and Gotelli (2001) studied pit building behaviour of *Myrmeleon immaculatus* larvae, and how temperature influences the pit building of the species. The optimum temperature of *Myrmeleon obscures* and connection between the temperature and life cycle were analysed by Bakoidi *et al.*, (2019). The influence of soil temperature and soil illumination in *M. Immaculatus* larvae were studied by Klein (1982).

There are only limited studies were present regarding the altitude and pit building. Bozdogan *et al*., (2013) observed some peculiarities of *Myrmeleon formicarius* in forest areas and non forest areas of Kahramanmaras in Turkey.

2.2.4. Soil preference

The main characteristic features of soil include soil temperature, soil moisture and soil texture. The different species of genus *Myrmeleon* was used for most of the studies and the pit building of *Myrmeleon pictifrons* in moisture condition and different grain size were studied by Kitching (1984). Sand preference study

reveals the preference of find sand (Lucas, 1986) for pit building in *Myrmeleon sp*. Instability of sandy soil was studied by Halloran *et al.*, (2000) and the antlion larvae (*Myrmeleon crudelis)* considered as bioindicator of soil stability. Farji-Brener (2003) determined whether the soil condition or ant acacia clearings influence the pit trap construction and the results shows that soil condition is most significant than ant acacia. The pit building strategy in different conditions like sand depth, soil type and thermal conditions were studied byAlcalay *et al.*, (2014). Maoge *et al*., (2014) analysed the chemical composition of the media and the role in the pit building of antlion larvae. In a three year survey, *Myrmeleon quinquimaculatus*, *M. obscures* and *Hagenomya tristicis* are the most abundant species of Northern part of Cameroon.

2.3. RESEARCH METHODOLOGY

2.3.1. Study Area- Kerala

Kerala lies between northern latitude of 8º.17'.30"N and 12º.47'.40"N and east longitudes 74º.27'.47"E and 77º.37'.12"E (Map-1). Geographically Kerala is situated between Arabian sea to the west and the Western ghats to the east. Physiographically the land has three regions, such as lowlands (0-7.5m altitude), midlands (7.5-75 m altitude) and highlands (>75 m altitude). The forest types in Kerala includes dry deciduous, moist deciduous, semi evergreen, evergreen and shola forests (Champion and Seth, 1968). Ten types of soils were noted from Kerala such as red soil, laterite soil, coastal alluvial, riverine alluvial soil, greyish onattukara soil, brown hydromorphic soil, hydromorphic saline soil, acid saline soil, black soil and forest soil.

2.3.2. Meteorological data of Kerala

In Kerala, there are four seasons such as summer (March to May), South west monsoon (June to September), North east monsoon (October to December) and winter (January and February) and the average temperature and rainfall is given in Table 5. The mean maximum temperature is seen in the month of March in Kerala, which is about 33ºC. The minimum temperature noted in the month of July is about 28.5ºC. From January to March, the humidity varies from 35% to 71% in the state, and during the monsoon, it is about 85%. The South west monsoon (June to October) comprises the 70% of annual rainfall in Kerala (Table 6).

Table 5. Seasons in kerala with average temperature and rainfall

Seasons in Kerala	Average temperature		Average rainfall (mm)
	Max (ºC)	Min (ºC)	
Winter	28	18	25
Summer	36	32	135
South west monsoon	30	19	2250-2500
North east monsoon	35	29	450-500

Table 6. Average monthly rainfall in Kerala

Average monthly rainfall in Kerala												
Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Rain Fall (mm)	14.6	16.6	36.1	110.9	252.6	653.2	687.2	404.7	252.3	270.7	158.6	45.9

(ENVIS: Kerala state action plan on climate change, source: Economic review 2013-2018, IMD TVRM)

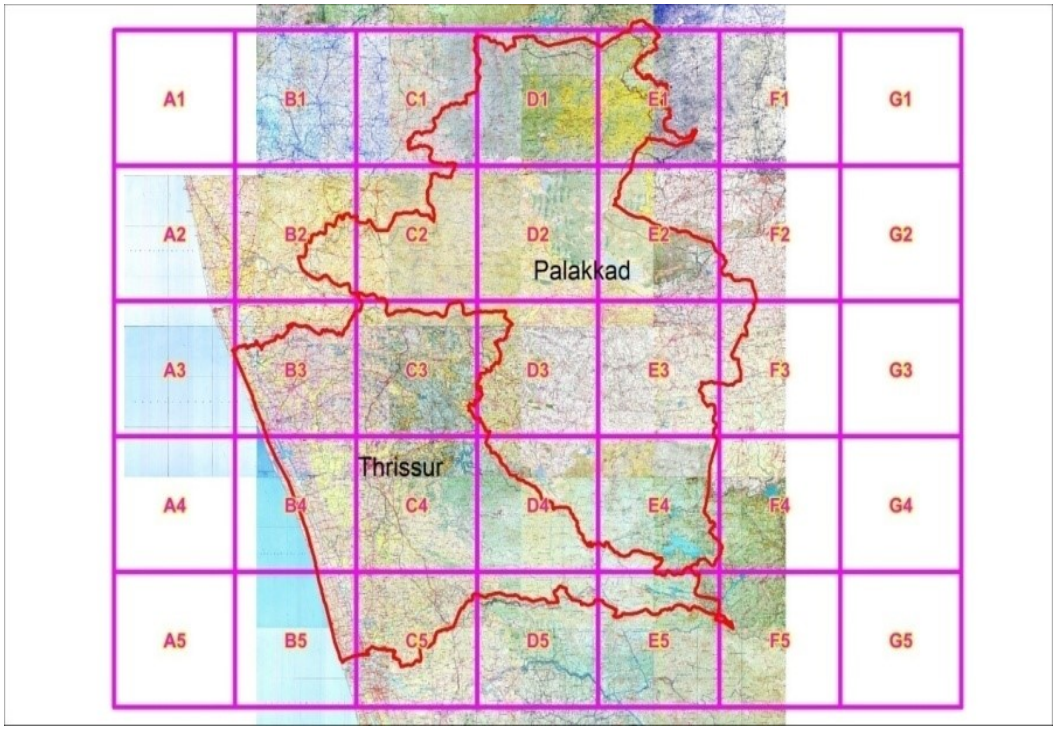
2.3.3. Sampling

A pilot study was carried out from June 2015 to December 2015 for identifying the presence of antlion species in Thrissur and Palakkad districts of Kerala. These two districts were divided into 20 grids and in each grid the area was set to 625 km^2 (25X25 km) (Map-2). Out of the 20 grids surveyed, 15 grids were randomly selected for the collection purpose. From this initial survey it was understood that the number of species and individuals were not sufficient for behavioural and ecological studies. Therefore it was decided to cover all districts for maximum species collection and identification of habitat.

Genus *Myrmeleon* is the common pit building antlion in India (176 species worldwide), and the study concentrated on Genus *Myrmeleon* and the behaviour study also done by using the common species of this genus. The collection of antlion was carried out from January 2016 to December 2019 and for behaviour study focussed on the pit building antlion species. Care was given to choose at least one study area in each district for the collection of antlion.

Random collection was done and the collection methods include visual encounter survey and active search due to the unavailability of previous studies regarding the habitat in southern India.

Map 1- Study Area- Kerala



Map 2- Map plotted for pilot study

2.3.4. Collection Techniques (Both adult and larvae)

Collection techniques adopted for the study includes handpicking, sweeping and light trap.

Handpicking: Antlion larvae were collected by handpicking or by using a spoon (Maoge *et al*., 2014) in the day time between 7 am to 4 pm. Larvae were scooped by the help of a spoon and it was transferred to a paper cup (diameter 6 cm and height 6 cm) filled with sand/soil.

Sweeping: This method was used to collect adult antlions during the day time. The bushes and plants in different localities were disturbed using a wooden stick. The antlions present were collected using a hand net.

Light trap: As the antlion adults are attracted to light during the early morning and evening periods, light traps were set up. Light traps consisted of a white cloth hung near a 9wt LED (Light emmiting diode) bulb. The presence of antlion adult was observed in active time (5.30 to 7.30 am and 6.30 to 8.30 pm) near light trap.

2.3.5. Killing and Preservation

The collected adults were killed using chloroform and spread on a spreading board. The spread, pinned and labelled specimens were stored in insect boxes for the purpose of identification. Specimens used for morphological studies were carefully taken and slides of legs, antennae and wings were prepared by mounting in DPX without staining.

2.3.6. Rearing of Antlion larvae

For rearing of antlion larvae, individuals were observed and collected after measuring the pit depth and diameter. Field collected individuals were brought to the laboratory and the morphometric measurements of larval body parts were taken. Measured individuals were transferred to a paper cup (diameter 6 cm and height 6 cm) filled with sand or dry soil (Liang *et al*., 2010; Guillette *et al*., 2009; Maoge *et al*., 2014). The paper cups were covered by using a cloth in order to avoid the escape of adult after emergence. The larvae were given one ant

(*Anoplolepis gracilipes*) per day as food. When the larva stops feeding, they are allowed to moult or pupate. After emergence of the adult, it was spread, dried and kept in the insect box for further studies.

2.3.7. Depository

The curated adult antlion specimens were deposited in the Research and Postgraduate Department of Zoology, St. Thomas' College (Autonomous), Thrissur) for further reference.

2.3.8. Identification of collected Specimens

The collected specimens of antlion adult and larvae were identified by using standard taxonomic keys and for substantiating the results molecular sequencing were performed (Stange, 2004 and Ghosh, 2000) by the following protocol.

2.3.8.1. DNA sequencing using universal primers of CO1

Genomic DNA Isolation: The genomic DNA isolation was done by using NucleoSpin® Tissue Kit (Macherey-Nagel).Tissue was placed in 1.5ml microcentrifuge tube. Added 180µl of T1 buffer and 25µl of Proteinase K and incubated at 56ºC in a water bath. Then added 5µl of Rnase A (100mg/ml) and incubated at room temperature for 5 minutes. Added 200µl of B3 buffer and incubated at 70ºC for 10 minutes. 210µl of 100% ethanol was added followed by vortexing. The mixture was pipetted in to NucleoSpin® Tissue column placed in a 2 ml collection tube and centrifuged at 11000xg for 1 minute. NucleoSpin® Tissue column transferred to a 2 ml tube and washed with 500µl of BW buffer. This step was repeated using 600µl of B5 buffer. NucleoSpin® Tissue column placed in 1.5ml tube and DNA eluted using 50µl of BE buffer.

Agarose Gel Electrophoresis for DNA Quality Check: 1µl of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH-8.0) was added to 5µl of DNA. Samples loaded to 0.8% agarose gel prepared in 0.5 X TBE (Tris-Borate-EDTA) buffer containing 0.5µg/ml ethidium bromides. Electrophoresis performed with 0.5X TBE as buffer at 75V. Gels were visualized in UV transilluminator (Genei) and the image captured under UV light using Gel documentation system (Bio-Rad).

PCR (Polymerase chain reaction) Analysis: PCR amplification was done in a 20 µl reaction volume containing 1X Phire PCR buffer (contains 1.5 mM $MgCl_2$), 0.2 mM each dNTPs (dATP, dGTP, dCTP and dTTP), 1 µl DNA, 0.2 µl PhireHotstart II dNA polymerase enzyme, 0.1mg/ml BSA and 3% DMSO, 0.5 M Betaine, 5pM of forward and reverse primers (Table 7). Amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems).

Table 7. Primers used for PCR

Target	**Primer Name**	**Direction**	**Sequence(5'→3')**
COX1	LCO	Forward	GGTCAACAAATCATAAAGATATTGG
	HCO	Reverse	TAAACTTCAGGGTGACCAAAAAATCA

PCR amplification profile

COX1

98ºC- 30 sec

98ºC-5 sec
45ºC- 10 sec
72ºC- 15 sec
} 10 cycles

98ºC- 5 sec
50ºC- 10 sec
72ºC- 15 sec
} 30 cycles

72ºC- 60 sec

4ºC- ∞

Agarose Gel electrophoresis of PCR products: The PCR products were checked in 1.2% agarose gels prepared in 0.5X TBE buffer containing 0.5 µg/ml ethidium bromide. 1 µl of 6X loading dye was mixed with 5 µl of PCR products and was

loaded and electrophoresis was performed at 75V power supply with 0.5X TBE as electrophoresis buffer for about 1-2 hours, until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard used was a 2-log DNA ladder (NEB). The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

ExoSAP-IT Treatment: ExoSAP-IT (GE Healthcare) consists of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer for the removal of unwanted primers and dNTPs from a PCR product mixture with no interference in downstream applications.

Five micro litres of PCR product is mixed with 2 µl of ExoSAP-IT and incubated at 37ºC for 30 minutes followed by enzyme inactivation at 80ºC for 15 minutes. Sequencing using BigDye Terminator v3.1: Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following manufactures protocol.

The PCR mix consisted of the following components:

PCR Product (ExoSAP treated)- 10-20 ng

Primer - 3.2 pM (either Forward or Reverse)(Table 7)

Sequencing Mix - 0.28 µl

DMSO - 0.30 µl

5x Reaction buffer - 1.86 µl

Sterile distilled water - make up to 10µl

The sequencing PCR temperature profile consisted of a 1st cycle at 96ºC for 2 minutes followed by 30 cycles at 96ºC for 30 sec, 50ºC for 40 sec and 60ºC for 4 minutes.

Post Sequencing PCR Clean up: Master mix I of 10µl milli Q and 2 µl 125mM EDTA per reaction and master mix II of 2 µl of 3M sodium acetate pH 4.6 and 50 µl of ethanol were prepared. 12µl of master mix I was added to each reaction containing 10µl of reaction contents and was properly mixed. 52 µl of master mix

II was added to each reaction. Contents were mixed by inverting and incubated at room temperature for 30 minutes. Spun at 14,000 rpm for 30 minutes. Decanted the supernatant and added 100 µl of 70% ethanol. Spun at 14,000 rpm for 20 minutes. Decanted the supernatant and repeated 70% ethanol wash. Decanted the supernatant and air dried the pellet. The cleaned up air dried product was sequenced in ABI 3500 DNA Analyzer (Applied Biosystems).

Sequence Analysis: The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond *et al*., 2010). The final sequence was run in BOLD systems (Barcode of Life data system) and NCBI BLAST (National Centre for Biotechnology Information, Basic Local Alignment Search Tool) and the maximum similarity showing species in the database was used to confirm the species. Phylogenitic trees (Neighbour tree without distance corrections) were prepared by using Clustal Omega.

2.3.9. Habitat

The latitude, longitude and the physical parameters of the study area was noted for understanding the habitat of antlion larvae. According to the data, antlion larval habitats were classified as abandoned area, human dwelling area, forest boundary area and riparian. The area in which no disturbance of animals and human being are considered as abandoned area. Mainly it includes abandoned buildings and land without the presence of human activity (under the shades of trees). The area near to houses, schools, bus stops etc are considered as human dwelling area. The areas up to 5 kms from forest boundaries were considered as forest area, which included abandoned areas, buildings, and shaded areas due to the difficulty of collecting antlion larvae inside the forests. River banks were considered as riparian habitat.

2.3.10. Morphometric Analysis-Larvae

Morphometric measurements of two species of genus *Myrmeleon* were made. *Myrmeleon hyalinus* and *Myrmeleon pseudohyalinus* were used for this study.

For this purpose the larval body length, larval body width, larval head length, larval head width, mandible length were calculated by using measurement tool of leica stereozoom research microscope (LEICA S8APO) (Plate 15) attached with digital camera (LEICA MC170HD). The statistical analysis of the data was performed in PAST software (3 & 4.2 versions).

In order to identify the relationship between the antlion larval size and pit size, observations were conducted in the sunshade of an abandoned house in Parli, Palakkad district, from April 1 - May 30, 2015. A natural population of *M. pseudohyalinus* larvae was selected for this experiment and the depth and diameter of the pits were noted without disturbing the antlion larvae. Pit diameters and depth were measured to the nearest millimeter. The soil temperature of the study area also recorded.

For understanding the correlation between the pit size and larval size in natural condition the following parameters were studied; pit depth, pit diameter, larval head length, larval head width, larval body length and larval body width. These above measurements were taken on site. The non-resident pits or abandoned pits of antlion larvae were also noted. The correlation between larval size and pit parameters were examined to understand whether its larvae used to predict larval instar.

2.3.11. Morphometric Analysis-Cocoon

The cocoons of genus *Myrmeleon* were collected by sieving the soil through a net from antlion habitats in which the presence of pit was noted. After the collection, it was labelled and stored in zip covers without any damage in the field itself.

The cocoons were examined and the presence /absence of pupa was determined by noting the emergence hole (Plate 14). Cocoon with hole indicated that the adult had emerged and escaped from the cocoon. The absence of emergence holes were indicated the presence of pupae inside cocoon and it was shifted to paper cups filled with dry soil for ensuring the emergence of the adult antlions. Cups were covered with nets and kept in laboratory under room temperature. The cocoon morphology was analysed by carefully measuring its diameter, weight, and circumference (Plate 16). The circumference was measured using a vernier

calliper and weight of cocoon was noted by using Shimadsu digital weighing balance.

2.3.12. Morphometric Analysis- Adult

The following morphometric measurements of *Myrmeleon pseudohyalinus* were taken for this study: length from head to the tip of abdomen, forewing length, forewing breadth, hindwing length, hindwing breadth and length of antennae. The length of the wings was measured from the base to the apex, and the width was taken as the maximum width perpendicular to the length measurement line (Pantaleoni *et al.*, 2010).

2.3.13. Physical Parameters

From each study area the physical characters of the habitat was noted by using a digital temperature and humidity checker and the soil temperature was noted by using a thermometer. Temperature, humidity, dew point, pressure, UV index, visibility and wind speed were noted for understanding the habitat character of antlion larvae by using gadgets and from weather.com. Pearson's correlation performed in Past3 software for statistical analysis and the significant level was set at 5%. Significant positive correlations are marked in blue and boxed. Significant negative correlations are marked in red and boxed in the correlation plot. Those not boxed are not significant.

2.3.14. Soil Texture Analysis

Soils samples were collected from selected habitats based on the availability. 100 grams of soil is needed for the texture analysis and again a 100 gram is needed for chemical analysis. So the samples collected from antlion inhabited area are taken for the above purpose in a zip cover and carried to Research lab (Plate 15). The collected soil samples were weighed and sorted for the collection of all the prey species from that area. After that 100 grams were packed tightly and the texture was analysed in the labs of IRTC (Integrated Rural Technology Centre), Mundur, Palakkad and Soil Science Department of KFRI (Kerala Forest Research Institure), Peechi, Thrissur. The methodology used for the analysis of texture was Hydrometer method.

Hydrometer method: It is based on the principle of dispersion and sedimentation techniques employed to a given weight of soil sample. Sedimentation refers to the settling rates of the dispersed particles in water, which is function of particle size and is governed by Stoke's law. Theoretically the hydrometer measures the density of soil suspension. In practice, an average density of the depth of the inserted hydrometer is taken. The hydrometer is based on the fact that the suspension at a given depth decreases as an initially homogeneous dispersed suspension settles. The rate of decrease in density, at any given depth, is directly related to the settling velocities of the particles, which in turn are related to their size. The hydrometer reading indicates that 4 minutes after sedimentation particles greater than 0.02 mm settle which after 2 hours, particles of size less than 0.002mm are left in soil suspension.

Procedure:-Weighed 50 g fine textured soil or 100 g coarse textured soil (>75-80% sand) which have been passed through a 2 mm sieve based on oven dry condition in to a beaker. Added 50 ml of 6% H_2O_2 (Hydrogen peroxide) and covered the beaker with a watch glass and placed it on a water bath until oxidation of organic matter is completed (indicated by the presence of effervescence), removed the beaker and cooled. After cessation of frothing transferred the contents in to a dispersing cup with about 400 ml of distilled water. Added to it 100 ml of calgon (a combination of sodium hexametaphosphate and sodium carbonate) solution. Stirred the suspension for 10 minutes by an electric stirrer. Transferred the suspension into a litre graduated cylinder and made up the suspension up to 1 litre mark with distilled water. Stopper the mouth of the cylinder and shaked vigorously upside down and back several times for about 1 minute. Placed the cylinder on a table and note the time immediately. Dipped the hydrometer in to the suspension and take the first reading after 4 minutes when particle >0.02mm have settled (Start inserting the hydrometer 10 seconds in advance of the reading time). Carefully removed the hydrometer and washed with distilled water and noted down the temperature of the suspension.

Note- The hydrometer is calibrated at 67^{o}F (19.4^{o}C). If the suspension temperature is above 67^{o}F, the correction is added, and if below, the correction is subtracted. The correction is equal to the difference between the experimental temperature and 67^{o}F multiplied by 0.2.

For conversion of ºF to ºC the following equation is used $\frac{C}{5} = \frac{F-32}{9}$

Kept the suspension undisturbed and dip the hydrometer again at the end of 2 hours after initial shaking was stopped. Now, the particles greater than 0.002 mm (sand+silt) have settled. Recorded the hydrometer reading. Calculated the percentage of sand, silt and clay and determined the textural class using ISSS textural triangle.

2.3.15. Soil Chemical Analysis

Though antlion larvae spent most of its life cycle in soil or sand, the chemical composition or chemical parameters may influence the larvae. For this purpose analysed the following chemical component of the antlion larvae inhabited soil. Soil pH, Electrical Conductivity, Organic Carbon, Available Phosphorus, Available Potassium, Available Calcium, Magnesium, Available Sulphur, Iron, Manganese, Zinc, Copper, Available Boron, Nitrogen and Chlorine were analysed and the various methodology used for the analysis were explained below.

2.3.15.1. Soil pH

It is a measure of hydrogen ion concentration of the soil water system and indicates whether the soil is acidic, neutral or alkaline in reaction.The reagents used as follows, Standard buffer solutions: Buffer solutions of pH 4.0, 7.0, 9.2 were prepared using commercially available buffer tablets. Dissolved the respective tablets in freshly prepared distilled water and made up the volume to 100 mL. Calibrated the pH meter using buffer solutions. The pH of soil is determined in 1:2.5 soil water suspensions. Took 10 g sample of soil sifted through 2 mm sieve in a 50 or 100 mL beaker. Add 25 mL of distilled water, stir well for about 5 minutes and keep for half an hour. Stir well again and take the reading using the pH meter.

2.3.15.2. Electrical conductivity (EC)

Electrical conductivity in soil water system is a measure of concentration of soluble salts and extent of salinity in the soil and is measured using a conductivity meter. The clear supernatant of 1:2.5 soil water suspension prepared for pH

measurement can be used for estimation of EC. Calibrated the conductivity meter using 0.01N KCl (Potassium chloride) solution prepared and determined the cell constant. Determined the conductivity of the supernatant liquid.

2.3.15.3. Organic carbon in soil (OC)

Soil organic matter has been defined as the organic fraction of soil, including plant, animal and microbial residues, fresh and at all stages of decomposition and the relatively resistant soil humus. However, soil organic matter estimate includes only those organic materials that accompany soil particles through a 2 mm sieve. Carbon is the chief element present in soil organic matter, and forms 48-58% of the total weight. Therefore organic carbon determinations are often used as a basis for estimation of organic matter.The principle behind this procedure was according to Schollenberger (1927) and the methodology used for the estimation was Walkley-Black Wet Digestion Method (Walkley, 1947)

The Procedure is as follows**,** Grinded soil upto pass through a 0.5 mm sieve and transferred a weighed sample (approximately 0.5 to 1 g soil) into a 500 ml wide mouth conical flask. Added 10 ml of 1*N* $K_2Cr_2O_7$ (Pottassium dichromate) and swirled the flask gently to disperse the soil in the solution. Then rapidly added 20 ml of concentrated H_2SO_4 (Sulfuric acid). Immediately swirled the flask gently until the soil and the reagents are mixed, then more vigorously for a total of one minute. Allowed the flask to stand on an asbestos sheet for about 30 minutes. Then added 200 ml of water to the flask. Added o-phenanthroline indicator and titrated the solution with 0.5*N* ferrous ammonium sulphate ($Fe(NH4)_2SO_4$). As the endpoint approaches, the solution takes on a greenish cast and then changes to a dark green colour. The ferrous ammonium sulphate was added drop by drop until the colour changes sharply from blue to red.

Calculation

$$\text{Organic carbon (\%)} = \frac{(\text{meq } K_2Cr_2O_7 - \text{meq } Fe(NH_4)_2SO_4) \times 0.003 \times 100 \times 1.3}{\text{Weight of soil}}$$

O C (%) = {10 x 1 – Titre Value(mL) x Normality of $Fe(NH_4)_2SO_4$} x 0.003 x 100 x 1.3

Weight of soil

O C (%) = {10 x 1 – Titre Value(mL) x Normality of $Fe(NH_4)_2SO_4$} x 0.39

Weight of soil

2.3.15.4. Available phosphorus (P)

Determination of plant available P in soil has two distinct phases – first, the extraction of plant available pool of phosphorus, present in soil, and second the quantitative determination of the P in the extract. The choice of a colorimetric method for determining P depends on the P concentration in the solution, the concentration of interfering substances in the solution to be analysed and the particular acid system involved in the analytical procedure. The molybdenum blue method is the most sensitive and widely used one for soil extracts containing small amounts of P. The available pool of P varies depending on the pH of the soil, reagents used for extraction of this pool also are different.

Procedure includes Extraction and Estimation by reduced molybdate blue colour methods. The Extraction: Weighed out 5 g of soil to a 100 mL conical flask and added 50 mL of Bray No.1 reagent (Bray and Kurtz, 1945) and shaked for exactly 5 minutes. Filtered through Whatman No.42 filter paper. To avoid interference of fluoride, 7.5 mL of 0.8 *M* boric acid (50 g of H_3BO_3 per litre) can be added to 5 mL of the extract if necessary. Estimated phosphorus in the extract by ascorbic acid method (Watanabe and Olsen, 1965).

Estimation by reduced molybdate blue colour method: Pipetted out 5 mL of the extract into a 25 mL volumetric flask and diluted it to approximately 20 mL. Added 4 mL of Ascorbic acid (1.056 g of ascorbic acid in 200mL of Ammonium paramolybdate). Made up the volume with distilled water and shaked the contents well. Read the intensity of colour after 10 minutes at 660 nm. The colour is stable for 24 hours and the maximum intensity develops within 10 minutes. The concentration of P in the sample is computed from the standard curve (Plot the concentration vs Absorbance curve on a graph paper)

Calculation

Available P (mg kg^{-1} soil) = µg P mL^{-1} of the aliquot x $\frac{50}{5}$ x 25

Available P (mg kg^{-1} soil) = $\frac{\text{Absorbance for sample}}{\text{Slope of Std. Curve}}$ x 50

Available P (kg ha^{-1} soil) = Available P (mg kg^{-1} soil) x 2.24

2.3.15.5. Available potassium (K)

A relatively small portion of the total K in soils is exchangeable (approximately 1%). Exchangeable K generally ranges from <100 to 2000 µg mL^{-1} or more when compared with total K values which is in the order of 1 to 2%. Water soluble K seldom exceeds a few parts per million except in the case of certain saline soils.

The Procedure is as follows**,** Extraction: Shaked 5 g of soil with 25 mL of neutral normal ammonium acetate for 5 minutes and filtered immediately through a dry Whatman No.42 filter paper. First few mL of the filtrate may be discarded. Potassium concentration in the extract is determined using flame photometer after necessary setting and calibration of the instrument.

Standard curve for potassium: Diluted measured aliquots from the standard solution using ammonium acetate solution to give concentrations of 5 to 20 µg mL^{-1} K. After attaching the appropriate filter and adjusting the gas and air pressure, set reading in the flame photometer as zero for the blank (ammonium acetate) and at 100 for 20 µg mL^{-1} K. The curve is obtained by plotting the readings against the different concentrations (5, 10, 15 and 20 µg mL^{-1}) of K. Fluctuation in gas and air pressure does not allow steady reading in the meter and must be taken care of.

Calculation

Available K (mg kg^{-1} soil) = µg K mL^{-1} of the aliquot x $\frac{25}{5}$

Available K (mg kg^{-1} soil) = µg K mL^{-1} of the aliquot x 5

Available K (kg ha^{-1}) = Available P (mg kg^{-1} soil) x 2.24

2.3.15.6. Available calcium and magnesium (Ca and Mg)

As in the case of potassium, exchangeable plus water soluble calcium and magnesium contribute to the plant available pool. The neutral normal ammonium acetate extracts the pools of calcium and magnesium also along with potassium and sodium.The principle is, the cations Ca^{2+}, Mg^{2+} and Na^{+} along with K^{+} appear to be completely exchangeable in the absence of excess of $CaCO_3$ (Calcium carbonate) by neutral normal ammonium acetate.

Extraction of available Calcium and Magnesium: Shaked 5 g of soil with 25 mL of neutral normal ammonium acetate for 5 minutes and filtered immediately through a dry Whatman No.42 filter paper. First few mL of the filtrate may be discarded.

Estimation of Calcium and Magnesium by Atomic Absorption Spectrophotometry: From the soil extract Ca and Mg can be estimated by Atomic Absorption Spectrophotometry (AAS). The chemical interference, resulting from the formation of stable compounds between Ca and Mg ions and the accompanying anions may reduce the absorption. This interference may be overcome by using a realising agent such as Lanthanum or Strontium.

Calculation

Available Ca/Mg (mg kg^{-1} soil) = µg Ca/Mg mL^{-1} of the aliquot x $\frac{25}{5}$

Available Ca/Mg (mg kg^{-1} soil) = µg Ca/Mg mL^{-1} of the aliquot x 5

2.3.15.7. Available sulphur (S)- (By $cacl_2$ Extraction)

Different reagents have been proposed for extracting plant available sulphur from the soil. These include water, salt solutions such as 0.15% $CaCl_2$ (Calcium chloride), 500 ppm P as $Ca(H_2PO_4)_2$ (Calcium phosphate) or KH_2PO_4 (Potassium dihydrogen phosphate) and acidic solutions such as 0.5 *N* ammonium acetate plus 0.25 *N* acetic acid and Bray No. 1. Generally phosphate solutions extract more sulphate sulphur from soils than can be extracted with water or salt solutions

because phosphate ions displace the adsorbed sulphate, which is known to be readily available to plants.

Extraction (Tabatabai, 1982): Shaked 10 g of air-dried processed soil with 50 mL of 0.15% $CaCl_2$ solution in a 250 mL conical flask for 30 minutes. Filtered the extract through Whatman No. 42 filter paper and estimated the sulphate content by turbidimetric procedure.

Preparation of standard curve: Pipetted out 0, 0.25, 0.5, 0.75, 1.0, 1.25, and 2.5 ml of standard sulphate solutions in seven different volumetric flasks (25 mL) and added 10ml of extracting solution (0.15% $CaCl_2$). Prepared fresh standards each time when a batch of sample is analysed. Added 1 g of $BaCl_2$ (Barium chloride) crystals to each flask and dissolve it. Add 1 mL of 0.25% gum acacia solution (Dissolved chemically pure gum acacia in hot water and filtered the hot solution through Whatman No.42 filter paper, cooled the filtrate and diluted it to 100 mL), made up the volume with distilled water and shake well. After the development of turbidity (Within 5-30 minutes), read the absorbance at 440 nm on a spectrophotometer. Draw the standard curve with absorbance on Y axis and concentration on X axis.

Turbidimetric estimation of Sulphur (Massoumi and Cornfield, 1963): Pipetted out10 mL of the soil extract into a volumetric flask (25 mL). Added 1 g of $BaCl_2$ crystals and allow it to dissolve. Added 1 mL of 0.25% gum acacia solution, made up the volume with distilled water and shake well. After the development of turbidity (Within 5-30 minutes), read the absorbance at 440 nm on a spectrophotometer.

Calculation

$$\text{Amount of sulphur (mg kg}^{-1}\text{ soil)} = \text{Concentration from the instrument} \times \frac{25}{10} \times \frac{50}{10}$$

$$\text{Amount of sulphur (mg kg}^{-1}\text{ soil)} = \frac{\text{Absorbance for the sample}}{\text{Slope of Std. Curve}} \times 12.5$$

2.3.15.8. Iron, manganese, zinc and copper (Fe, Mn, Zn and Cu)

The major categories of micronutrient extractants presently in use are dilute acids, and solutions containing chelating agents, such as DTPA or EDTA. Among the chelating agents, DTPA is the most commonly used one. The DTPA soil test, developed for near neutral and calcareous soil by Lindsay and Norvell (1978) illustrates the evolution of a soil test extractant from theoretical principles. The extracting solution consists of 0.005 *M* DTPA (Diethylenetriamine pentaacetate) and 0.01 *M* $CaCl_2.2H_2O$, (Calcium chloride dihyadrate) buffered at pH 7.3 by 0.1 *M* triethanolamine (TEA). The DTPA extractant offered the most favourable combination of stability constants necessary to simultaneously extract four micronutrient cations (Fe, Mn, Cu and Zn). The buffered pH and presence of soluble Ca^{2+} prevent excessive dissolution of calcium carbonate avoiding the release of unavailable micronutrients occluded by this solid phase. At pH 7.3, 70-80% of the buffering capacity provided by TEA has been consumed. Therefore use of DTPA extractant on acidic soils, will result in neutralisation of remaining buffer capacity and unpredictable extraction pH.

Estimation of Fe, Mn, Zn, and Cu in acid soils (pH < 6.5)

Extraction and estimation*:* Shaked 2 g of soil with 20 mL of 0.1 *M* HCl (Hydrochloric acid) for 5 minutes. Filtered through Whatman No. 42 filter paper. Collected the filtrate and estimated the contents of Fe, Mn, Zn and Cu using an Atomic Absorption Spectrophotometer.

Calculation

$$\text{Amount of micronutrient (mg kg}^{-1}\text{ soil)} = \frac{\text{Concentration from the instrument x 20}}{2}$$

$$\text{Amount of micronutrient (mg kg}^{-1}\text{ soil)} = \text{Concentration from the instrument x 10}$$

Estimation of Fe, Mn, Zn, and Cu in near neutral to alkaline soils (pH > 6.5)

Extraction and estimation: Shaked 10 g of soil with 20 mL of DTPA for 2 hours. Filtered through Whatman No. 42 filter paper. Collected the filtrate and estimated the contents of Fe, Mn, Zn and Cu using an Atomic Absorption Spectrophotometer.

Calculation

Amount of micronutrient (mg kg^{-1} soil) = $\frac{\text{Concentration from the instrument x 20}}{10}$

Amount of micronutrient (mg kg^{-1} soil) = Concentration from the instrument x 2

2.3.15.9. Available boron (B) - (Hot-water soluble Boron (Gupta, 1967))

Although there are a variety of chemical tests for predicting crop response to boron, the hot water extraction procedure developed by Gupta (1967) is the easiest method.

Extraction and Estimation: Weighed 20 g of air-dried processsed soil in a 250 mL quartz or other boron-free conical flask and added 40 mL distilled water. Added 0.5 g of activated charcoal and boiled for 5 minutes on a hot plate, filter immediately through Whatman No.42 filter paper. Cooled the contents to room temperature and transferred 1 mL aliquot of blank, diluted boron standard, or sample solution into 10 mL polypropylene tubes**.** Added 2 mL of buffer and mixed. Added 2 mL of azomethine-H reagent (0.45 g of azomethine-H dissolved in 100 ml of 1% L-ascorbic acid solution), mix, and after 30 minutes, read the absorbance at 420 nm on a spectrophotometer. Prepared a standard curve plotting B concentrations (0 to 10 µg B mL^{-1}) on X-axis and absorbance on Y-axis.

Calculation

Amount of B in soil (mg kg^{-1} soil) = $\frac{\text{Absorbance reading}}{\text{Slope from curve}} \times \frac{40}{20}$

Amount of B in soil (mg kg^{-1} soil) = $\frac{\text{Absorbance reading}}{\text{Slope from curve}} \times 2$

2.3.15.10. Available Nitrogen (N)

Nitrogen in the soil sample was determined by Alkaline permanganate method. Weighed and transferred 20 g of soil in to a distillation flask. Added 30ml of distilled water just to moist the soil and 1 ml of liquid paraffin or 1 g of paraffin wax (to avaid frothing). Added few pieces of glass beads also to avoid bumping. Added 100 ml of freshly prepared 0.32% $KMnO_4$ (Potassium permanganate) and 100 ml of 2.5% NaOH (Sodium hydroxide) to the soil in the distillation flask. Kept a 100 ml beaker containing approximately 20 ml of 2% boric acid with double indicator below the delivery end of the condenser in the distillation set. Distilled the contents at a steady rate and collected the liberated ammonia in boric acid. Continued the distillation until the release is free of ammonia and about 30 ml of distillate is collected. Titrated the ammonia collected in boric acid with N/50 H_2SO_4.

Calculation

Weight of the soil taken= 20g

Volume of N/50 H_2SO_4 consumed=X ml (titre value)

1 ml of N/50 H_2SO_4= 0.00028 gN

X ml of N/50 H_2SO_4= 0.00028 x XgN

Therefore Nitrogen present in Kg/ha= $\frac{0.00028(X)2x10^6}{20}$

2.3.15.11. Available Chlorine (Cl)

Chloride determination is based on the formation of nearly insoluble silver salts. Silver nitrate in presence of potassium chromate indicator is used for precipitating Cl^-. Pipetted out 50 ml aliquot from the same soil-water extract or 5 ml of the filtered water sample. Added 5-6 drops of K_2CrO_4 indicator and titrated the solution with 0.02 (N) AgNo3 (Silver nitrate) solutions with stirring until the first reddish brown tinge appears. The ml of $AgNo_3$ required corresponds to the amount of chloride present.

2.3.16. Seasonal Adaptability and Habitat choice

The natural habitat of antlion larvae (Genus *Myrmeleon*) is mainly terrestrial in nature. Though antlion larvae inhabiting in the soil/sand, natural calamity like rain or flood affects more quickly than drought/high temperature condition. Experiments were conducted for analyzing the seasonal adaptability of soil inhabiting antlion larvae in the rainy condition and also analyzed the temperature tolerance or preferred soil temperature.

M.pseudohyalinus larvae were collected from soil without causing any damage. The second and third instar larvae were transferred to plastic trays (30X25cm) filled with loose soil (5 cm thickness). Five larvae were released in each tray and allowed to make its pits. Then larvae were given the rainy condition by spraying water in required quantity and noted the behaviour of the larvae and number of days taken for pit rebuilding. 20 ml of water was used for each spray and 3 experiments were performed such as two sprays per day, four sprays per day and six sprays per day in regular intervals (Freire and Lima, 2019.). After each spray the temperature of soil was noted and the pit building temperature was identified. The experiment repeated 10 times to decrease the bias.

Table 8. Water Sprays given and the time interval

	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml
Two sprays	8 am	8 pm				
Four sprays	8 am	12 am	4 pm	8 pm		
Six sprays	7 am	9.30 am	12 pm	2.30 pm	5 pm	7.30 pm

The quantity of water sprayed in a day in two sprays condition, four spray condition and six spray conditions were 40 ml, 80 ml and 120 ml respectively. Also conducted an experiment with wet and dry condition in a single tray. Tray (30X25 cm) filled with dry soil with 5 cm thickness, and partitioned it to two equal halves by using a cardboard. Then one part sprayed with 20 ml of water and the other side remained in the dry condition. After removing the cardboard five

larvae released in the centre of the tray and pit building behaviour was noted. Repeated the experiment in ten times.

2.3.17. List of abbreviations used

A- Attack

A D-Adult Antlion

AA- Abandoned area

AN-Antennae

CD-Cocoon diameter

CW- Cocoon weight

E- Emergence

F- Feeding

FB- Forest boundaries

FWB- Forewing breadth

FWL- Forewing length

G- Grooming

H- Holding

HDA-Human dwelling area

HR- Head roll

HTA- Head to the tip of abdomen

HWB- Hind wing breadth

HWL- Hind wing length

JS- Jaw set

KNR-Kannur

LBL-Larval body length

LBW-larval body width

LHL-Larval head length

LHW-larval head width

L-Larvae

Max-Maximum

Min-Minimum

ML-Mandible length

MLPM- Malappuram

PB- Prey beating

PC- Pit clearing

PD-Pit diamter

PKD-Palakkad

PR- Prey clearing

PTMA- Pathanamthitta

PT-Pit Depth

Q- Quiescence

RB- River banks/Riparian

S- Submergence

TCR- Thrissur

TVRM- Thiruvanathapuram

WYND-Wayanad

2.4. RESULTS

A total of 64 adults (31 collected as adult and the remaining emerged from larval rearing), 315 larvae and 75 cocoons were collected from the study areas all over Kerala. Specimens were grouped under species using DNA barcoding. For this purpose one or two specimens from each study area were kept in absolute alcohol before the specimen was killed.

2.4.1. Collection- Sampling sites

The 68 study sites which were surveyed all over Kerala for identifying the presence of pit building antlion larvae genus *Myrmeleon* are provided in Table 9. Palakkad (25 sites), Thrissur (12 sites), Thiruvananthapuram (3 sites), Kozhikode (5 sites), Ernakulam (4 sites), Wayanad (6 sites), Malappuram (6 sites), Kannur (1 site), Idukki (1 site), Kottayam (1 site), Kollam (1 site), Alappuzha (1 site), Kasargod (1 site) and Pathanamthitta (1 site) and the larvae were collected from Fifty study areas of kerala except Kozhikode district (Plate 5, 6 and 7).

Table 9. Visited study sites for antlion collection in Kerala

Sl No.	Name of place	Latitude	Longitude
1	Parli	10°47’36”N	76°33’52”E
2	Edathara	10°47’29”N	76°33’58”E
3	Pezhumpara	10°34’41”N	76°36’13”E
4	Murukkumpara	10°49’62”N	76°32’94”E
5	Prakruthigramam	10°29’52”N	76°45’24”E
6	Tagore theatre	8◦50’34”N	76°96’08”E
7	Kanalpalam	10°70’51”N	76°41’26”E
8	Walayar	10°84’28”N	76°83’88”E
9	Pattikkad	10°54’96”N	76°33’52”E
10	Sarovaram park	11°26’86”N	75°79’27”E
11	Nedumbasserry	10°16’79”N	76°39’78”E
12	Athani	10°15’30”N	76°46’21”E
13	Sulthan Bathery	11°39’38”N	76°15’04”E
14	Kadamanchira	11°41’10”N	76°15’31”E

15	Thiruvizhamkunnu	11º02’23”N	76º22’16”E
16	Bengalow kunnu	11º17’17”N	76º14’17”E
17	Dhoni temple	10º51’11”N	76º37’29”E
18	Soochippara	11º50’95”N	76º16’06”E
19	Meenvallam	10º55’24”N	76º33’51”E
20	Mukkali	11º66’84”N	75º55’88”E
21	Conoly	11º26’53”N	76º20’69”E
22	Poabs Tea factory	10º32’49”N	76º42’05”E
23	Kakkayam	11º54’73”N	75º89’26”E
24	Thusharagiri	11º28’22”N	76º03’14E
26	Poomala	10º36’00”N	76º14’35”E
27	Ezhattumugham	10º17’40”N	76º27’09”E
28	Kuruva	11º50’48”N	76º04’20”E
29	Thumboormuzhi	10º17’44”N	76º27’33”E
30	Vettilappara	10º29’22”N	76º51’49”E
31	Meenkara	10º62’45”N	76º80’43”E
32	Kiriyathupara	11º53’81”N	75º89’51”E
33	Ottappalam river	10º46’22”N	76º22’45”E
34	Parli Manamthody	10º47’33”N	76º33’51”E
35	Wadakkancherry	10º39’52”N	16º15’05”E
36	Nedupuzha	10º29’34”N	76º12’46”E
37	Kodungallur	10º13’20”N	76º12’09”E
38	Vellayani	8º25’53”N	76º59’09”E
39	Brennan college campus	11º46’41”N	75º28’07”E
40	Thennal resort, Kattikulam	11º50’26”N	76º05’05”E
41	Nilambur Dippo	11º16’44”N	76º15’11”E
42	Irrigation office	11º16’18”N	76º13’43”E
43	Idimuzhikkal	11º09’48”N	75º52’39”E
44	Marthoma college, Tiruvalla	9º24’02”N	76º35’02”E
45	Moyan modal school	10º46’46”N	76º39’17”E
46	Vettikkattiri	10º43’53”N	76º16’56”E
47	Kanniyampuram	10º46’14”N	76º21’26”E

48	Kinavallur	10º48’32”N	76º33’56”E
49	Meenakshivilas	11º65’53”N	75º96’44”E
50	Thathamangalam	10º67’72”N	76º71’63”E
51	Thiruvalathur	10º74’25”N	76º68’78”E
52	Asarikkadu	10º50’94”N	76º32’55”E
53	Ramakrishnappadi	10º46’07”N	76º26’27”E
54	Melarankode quarters	8º29’12”N	76º57’57”E
55	Tanur	10º98’20”N	75º87’54”E
56	Kanimangalam	10º48’61”N	76º20’88”E
57	Vandithavalam	10º65’91”N	76º74’23”E
58	Mananchira square	11º25’37”N	75º77’64”E
59	Suvarnodyanam	10º14’99”N	76º36’90”E
60	Padur	10º65’52”N	76º46’21”E
61	CPCRI, Chowki	12º31’40”N	74º58’06”E
62	Nangiarkulangara	9º25’90”N	76º46’35”E
63	Kollam Railway station	8º53’09”N	76º35’42”E
64	Ettumanur	9º40’14”N	76º33’24”E
65	Vadakkencherry	10º35’32”N	76º29’05”E
66	Maneed	9º90’55”N	76º45’78”E
67	Adimali	10º03’02”N	76º87’84”E
68	Kulappulli	10º78’75”N	76º27’98”E

2.4.2. Identification

For identifying the presence of different antlion species in Kerala, both adult and larvae were collected. The specimens were identified by the help of both classical and molecular taxonomy. Two species namely *Myrmeleon pseudohyalinus* and *Myrmeleon hyalinus* of pit building antlion larvae were observed in this study. *Myrmeleon pseudohyalinus* was used for the ecology and behaviour studies as more specimens of this species were collected.

Plate 5

Wayanad

Thrissur

Palakkad

Malappuram

Plate 6

Kannur

Pathanamthitta

Thiruvanathapuram

Kollam

Plate 7

Ernakulam

Kasargod

Idukki

Alappuzha

Kottayam

Plate 8

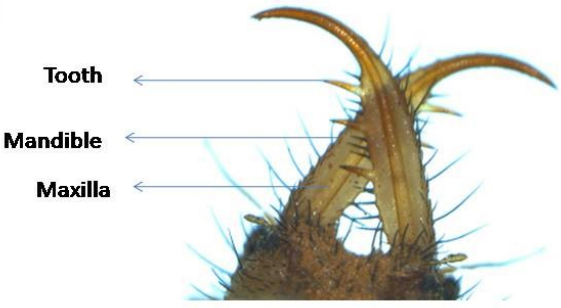
A

B

C

Fig. 1 A-C. General morphology of *M. pseudohyalinus* larvae A. Dorsal view, B. Ventral View, C. Abdomen

D

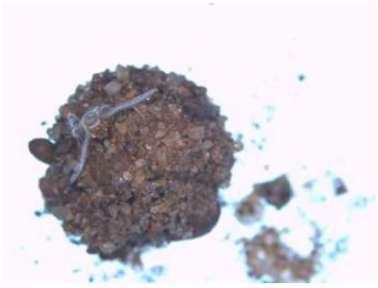


E

Fig 2. D . Dorsal portion of head, E. Mouth parts

Plate 9

F



G

H

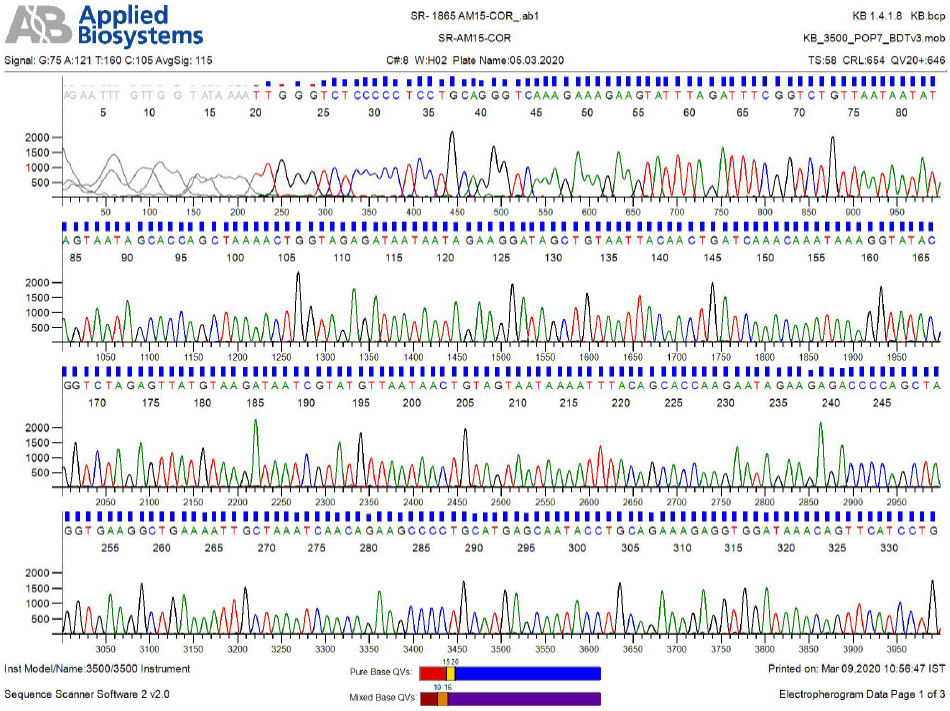
I

Fig. F-Exuviae, G- Cocoon with Exuviae, H- Adult, I- Tip of Abdomen

Adult- *M. Pseudohyalinus*

Plate 10

Sequence result of *Myrmeleon pseudohyalinus* collected from Adimali (Reverse Lap)



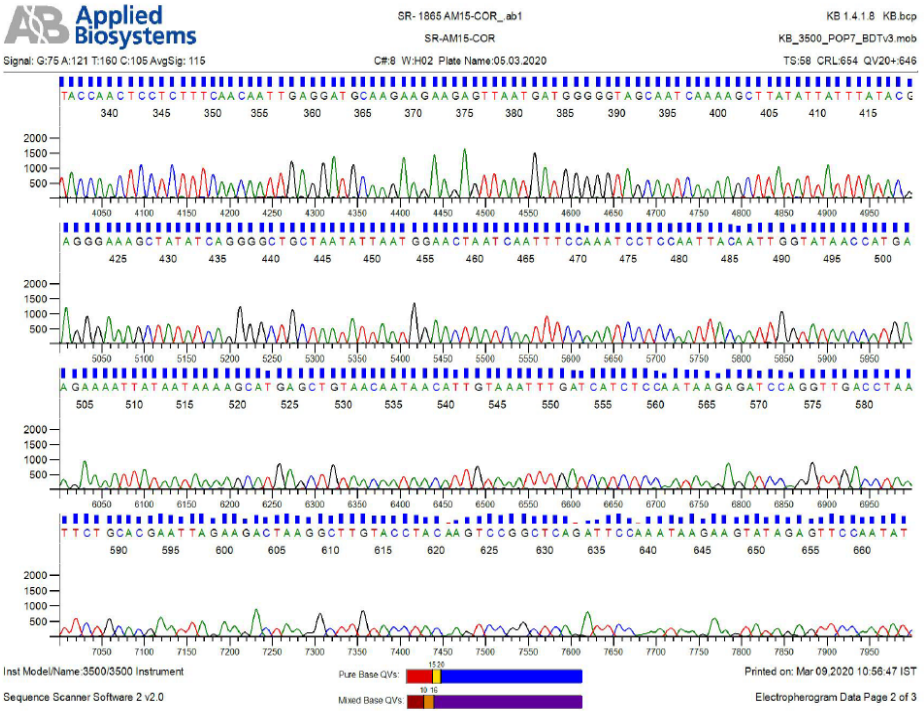
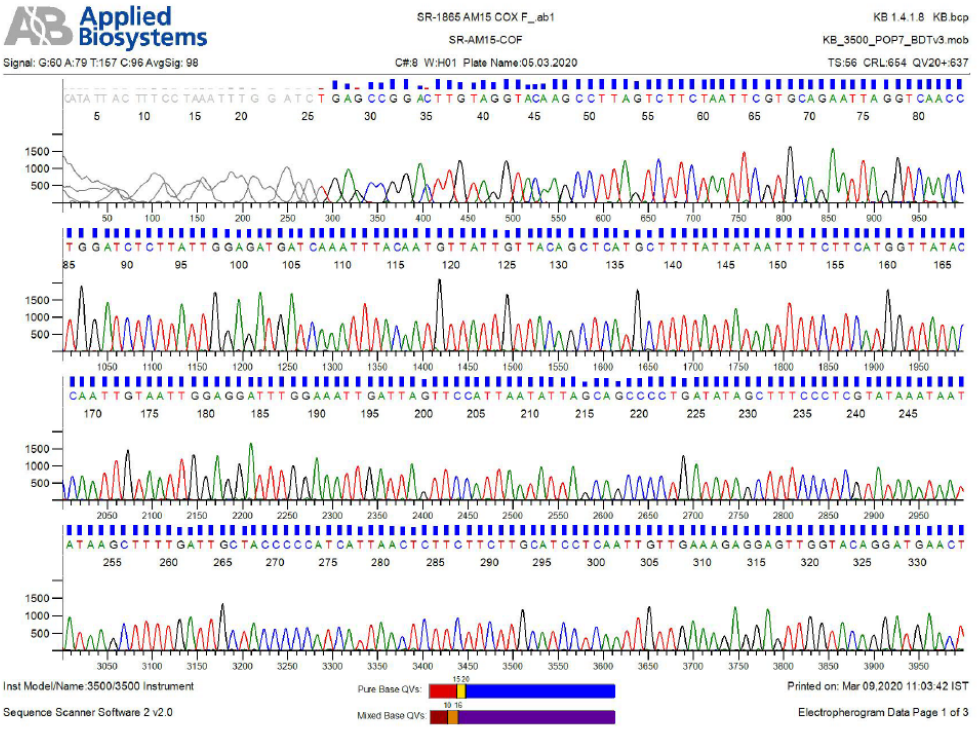


Plate 11

Sequence result of *Myrmeleon pseudohyalinus* collected from Adimali (Forward Lap)



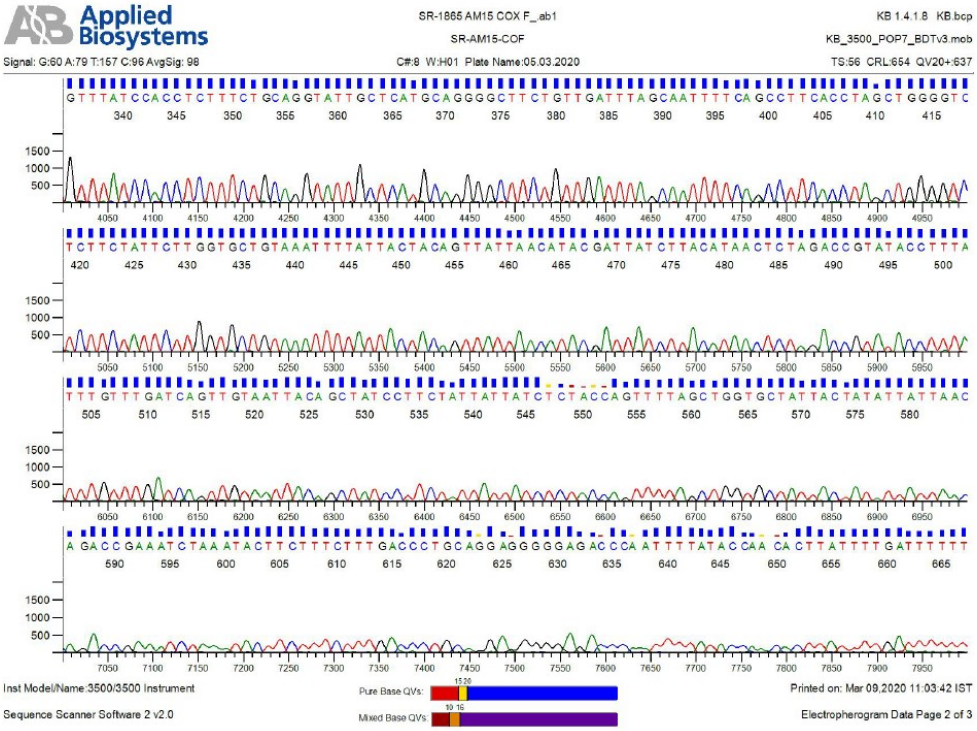
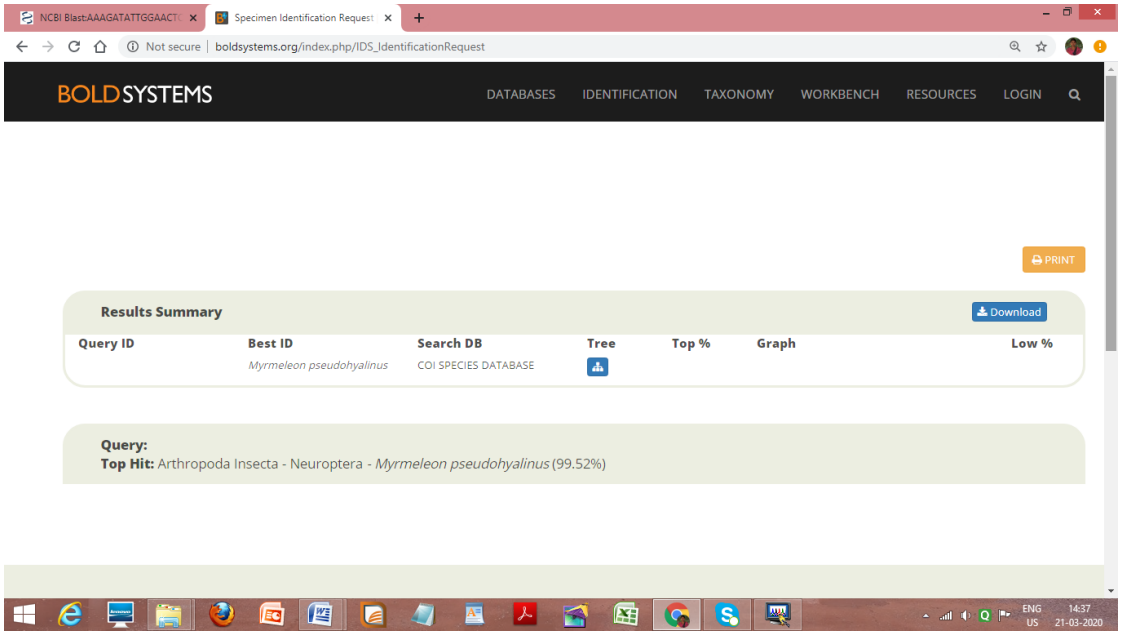


Plate 12

BOLD system result of *M.pseudohyalinus*



M. pseudohyalinus was collected from 9 study areas coming under 4 districts of Kerala. The detailed localities are specified in Table 10 and the general morphology of the larvae, cocoon, adult and exuviae are given in Plate 8 and 9.

Table 10. The collection sites of *M. pseudohyalinus*

Sl No	Locality	District	Latitude	Longitude
1	Meenavallam (L)	Palakkad	10º55'24"N	76º33'51"E
2	Dhoni (L)	Palakkad	10º51'11"N	76º37'29"E
3	Canoly (L)	Malappuram	11º26'53"N	76º20'69"E
4	Mukkali (L)	Palakkad	11º66'84"N	75º55'88"E
5	Kalady (AD)	Ernakuam	10º11'46"N	76º47'77"E
6	Kulappulli (L)	Palakkad	10º78'75"N	76º27'98"E
7	Vandithavalam (AD)	Palakkad	10º65'91"N	76º74'23"E
8	Maneed (L)	Ernakulam	9º90'55"N	76º45'78"E
9	Adimali (L)	Idukki	10º03'02"N	76º87'84"E

The DNA of nine samples of *M. pseudohyalinus* was extracted and amplified using standard methodology. The FASTA formats of DNA sequence of the species is described (Table 11) and chromatogram is given (Plate 10 and 11). Multiple sequence alaignment of sequences (Table 14) were performed in CLUSTAL O (1.2.4) and a phylogenetic tree was made (Fig. 1). The sequence was run in BOLD systems for the identification and confirmation. Also a phylogenetic tree was made with *M. pseudohyalinus* sequence and related species from NCBI (Fig. 2). From the tree it is clear that *M. formicarius, M. fasciatus, M. obscurus* and *M. quinquemaculatus* are species with common ancestry and *M. carolinus* and *M. crudelis* are another group of species with common ancestry. The remaining *M. mariaemathildae*, *M. caliginosus, M. hyalinus* and *M. pseudohyalinus* belongs to a common stalk.The sequence showed 99.52% similarity with the already existing sequence in the database (Plate 12). The sequence information of different populations of *M. pseudohyalinus* was given in Table 12.

Table 11. FASTA formats of *Myrmeleon pseudohyalinus* sequence of different populations

>AM05-Meenavallam ACCCCCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGAACTGTTTATCCACCTC TTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAGCAATTTTCAGCCTTCACCTAGCTGGGGTTTCTTCTATT CTTGGTGCTGTAAATTTTATTACTACAGTTATTAACATACGATTATCTTACATAACTCTAGACCGTATACCTTTATTTGT TTGATCAGTTGTAATTACAGCTATCCTT
> AM06-Dhoni CTGGTCAACAAATCATAAAAGATTATTGGAACTCTATACTTCTTATTTGGAATCTGAGCCGGACTTGTAGGTACAAGCCT TAGTCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCTCTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAG CTCATGCTTTTATTATAATTTTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATA TTAGCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCCCCATCATTAACTCTTCTTCT TGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGAACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAG GGGCTTCTGTTGATTTAGCAATTTTCAGCCTTCACCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACT ACAGTTATTAACATACGATTATCTTACATAACTCTAGACCGTATACCTTTATTTGTTTGATCAGTTGTAATTACAGCTAT CCTTCTATTATTATCTCTACCAGTTTT
>AM07-Canoly TTATTTGGAATCTGAGCCGGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCTCT TATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATTTTCTTCATGGTTATACCAATTG TAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATATTAGCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATA AGCTTTTGATTACTACCCCCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGAAC TGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAGCAATTTTCAGCCTTCACCTAGCTG GGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACTACAGTTATTAACATACGATTATCTTACATAACTCTAGACCGT ATACCTTTATTTGTTTGATCAGTTGTAATTACAGCTATCCTTCTATTATTATCTCTACCAGTTTTAGCTGGTGCT
>AM08-Mukkali ATAAAGATATTGGAACTCTATACTTCTTATTTGGAATCTGAGCCGGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGT GCAGAATTAGGTCAACCTGGATCTCTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTAT AATTTTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATATTAGCAGCCCCTGATA TAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCCCCATCATTAACTCTTCTTCTTGCATCCTCAATTGTT GAAAGAGGAGTTGGTACAGGATGAACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTT AGCAATTTTCAGCCTTCATCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACTACAGTTATTAACATAC GATTATCTTATATAACTCTAGACCGTATACCTTTATTTGTTTGATCAGTTGTAATTACAGCTATCCTTCTATTATTATCT CTACCAGTTTTAGCTGGTGCTATTACTATATTATTAACAGATCGAAATCTAAATACTTCTTT
>AM09-Kalady GGTCAACAAAATCATAAAGATATTGGAACTCTATACTTCTTATTTGGAATCTGAGCCGGACTTGTAGGTACAAGCCTTAG TCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCTCTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTC ATGCTTTTATTATAATTTTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATATTA GCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCCCCATCATTAACTCTTCTTCTTGC ATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGAACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGG CTTCTGTTGATTTAGCAATTTTCAGCCTTCATCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACTACA GTTATTAACATACGATTATCTTATATAACTCTAGACCGTATACCTTTATTTGTTTGATCAGTTGTAATTACAGCTATCCT TCTATTATTATCTCTACCAGTTTTAGCTGGTGCTATTACTATATTATTAACAGATCGAAATCTAAATACTTCTTTCTTT
>AM12-Kulappulli TCTATACTTCTTATTTGGAATCTGAGCCGGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGGTCAAC CTGGATCTCTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATTTTCTTCATGGTT ATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATATTAGCAGCCCCTGATATAGCTTTCCCTCGTAT AAATAATATAAGCTTTTGATTACTACCCCCATCATTGACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTA CAGGATGAACTGTTTACCCACCTCTTTCTGCAGGTATTGCTCATGCAGGAGCTTCTGTTGATTTAGCAATTTTTAGCCTT CATCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACTACAGTTATTAACATACGATTATCTTATATAAC

TCTAGATCGTATACCTTTATTTGTTTGATCAGTTGTAATTACAGCTATCCTTCTATTATTATCTTTACCAGTTTTAGCTG
GTGCTATTACTATATTATTAACAGATCGAAATCTAAATACTTCTTTCTTTGACCCTGCAGGAGGGGGGGACCC

>AM13-Vandithavalam

GGAACTCTATACTTCTTATTTGGAATCTGAGCCGGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGG
TCAACCTGGATCTCTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATTTTCTTCA
TGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATATTAGCAGCCCCTGATATAGCTTTCCCT
CGTATAAATAATATAAGCTTTTGATTACTACCCCCATCATTGACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGT
TGGTACAGGATGAACTGTTTACCCACCTCTTTCTGCAGGTATTGCTCATGCAGGAGCTTCTGTTGATTTAGCAATTTTTA
GCCTTCATCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACTACAGTTATTAACATACGATTATCTTAT
ATAACTCTAGATCGTATACCTTTATTTGTTTGATCAGTTGTAATTACAGCTA

>AM14-Maneed

AAAGATATTGGAACTCTATACTTCTTATTTGGAATCTGAGCCGGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGC
AGAATTAGGTCAACCTGGATCTCTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAA
TTTTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATATTGGCAGCCCCTGATATA
GCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCCCCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGA
AAGAGGAGTTGGTACAGGATGAACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAG
CAATTTTCAGCCTTCACCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACTACAGTTATTAACATACGA
TTATCTTACATAACTCTAGACCGTATACCTTTATTTGTTTGATCAGTTGTAATTACAGCTATCCTTCTATTATTATCTCT
ACCAGTTTTAGCTGGTGCTATTACTATATTATTAACAGACCGAAATCTAAATACTTCTTTCTTTGACCCTGCAGGAGGGG

>AM15-Adimali

AAAGATATTGGAACTCTATACTTCTTATTTGGAATCTGAGCCGGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGC
AGAATTAGGTCAACCTGGATCTCTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAA
TTTTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATATTAGCAGCCCCTGATATA
GCTTTCCCTCGTATAAATAATATAAGCTTTTGATTGCTACCCCCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGA
AAGAGGAGTTGGTACAGGATGAACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAG
CAATTTTCAGCCTTCACCTAGCTGGGGTCTCTTCTATTCTTGGTGCTGTAAATTTTATTACTACAGTTATTAACATACGA
TTATCTTACATAACTCTAGACCGTATACCTTTATTTGTTTGATCAGTTGTAATTACAGCTATCCTTCTATTATTATCTCT
ACCAGTTTTAGCTGGTGCTATTACTATATTATTAACAGACCGAAATCTAAATACTTCTTTCTTTGACCCTGCAGGAGGGG
GAGA

Table 12. The Sequence information of different populations of *Myrmeleon pseudohyalinus*

Sl no	Population	Bp length	Base count				AT content (%)	GC Content (%)
			A	T	G	C		
1	Meenvallam	268	105	62	57	44	62.31	37.69
2	Dhoni	587	229	154	94	110	65.25	34.75
3	Canoly	555	219	139	104	93	64.50	35.50
4	Mukkali	622	247	165	112	98	66.24	33.76
5	Kalady	639	252	171	116	100	66.20	33.80
6	Kulappulli	633	161	249	107	116	64.77	35.23
7	Vandithavalam	532	137	209	89	97	65.04	34.96
8								

	Maneed	640	166	247	107	120	64.53	35.47
9	Adimali	644	167	246	111	120	64.13	35.87

Seasonal abundance of *Myrmeleon pseudohyalinus* larvae were noted from January 2016 to December 2017 from three spots of Parli, Palakkad and it was tabulated such a way that the presence of larval pits noted (Percentage of occurrence was calculated by the number of *M.pseudohyalinus* larvae identified divided by total number of antlion larvae multiplied by hundred) and given in Table 13. The highest number of larvae and pits were present in the month of January to March followed by April to June (2016 and 2017).

Table 13. Seasonal occurance of *M. pseudohyalinus* larvae

Sl. No.	Months (2016 & 2017)	Percentage of occurance of *Myrmeleon pseudohyalinus*
1	January to March	44%
2	April to June	36%
3	July to September	2%
4	October to December	18%

Table 14. CLUSTAL O (1.2.4) multiple sequence alignment

```
AM15-Adimali            ------------------AAAGATATTGGAACTCTATACTTCTTATTTGGAATCTGAGCC
          42
AM14-Maneed             ------------------AAAGATATTGGAACTCTATACTTCTTATTTGGAATCTGAGCC
          42
AM07-Canoly             ------------------------------------------TTATTTGGAATCTGAGCC
          18
AM05-Meenavallam        ------------------------------------------------------------
          0
AM06-Dhoni              CTGGTCAACAAATCATAAAAGATTATTGGAACTCTATACTTCTTATTTGGAATCTGAGCC
          60
AM12-Kulappulli         ---------------------------------TCTATACTTCTTATTTGGAATCTGAGCC
          28
AM08-Mukkali            ----------------ATAAAGATATTGGAACTCTATACTTCTTATTTGGAATCTGAGCC
          44
AM09-Kalady             ---GGTCAACAAAATCATAAAGATATTGGAACTCTATACTTCTTATTTGGAATCTGAGCC
          57
AM13-Vandithavalam      ---------------------------GGAACTCTATACTTCTTATTTGGAATCTGAGCC
          33


AM15-Adimali            GGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCT
          102
AM14-Maneed             GGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCT
          102
AM07-Canoly             GGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCT
          78
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AM05-Meenavallam        ------------------------------------------------------------
          0
AM06-Dhoni              GGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCT
          120
AM12-Kulappulli         GGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCT
          88
AM08-Mukkali            GGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCT
          104
AM09-Kalady             GGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCT
          117
AM13-Vandithavalam      GGACTTGTAGGTACAAGCCTTAGTCTTCTAATTCGTGCAGAATTAGGTCAACCTGGATCT
          93


AM15-Adimali            CTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATT
          162
AM14-Maneed             CTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATT
          162
AM07-Canoly             CTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATT
          138
AM05-Meenavallam        ------------------------------------------------------------
          0
AM06-Dhoni              CTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATT
          180
AM12-Kulappulli         CTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATT
          148
AM08-Mukkali            CTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATT
          164
AM09-Kalady             CTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATT
          177
AM13-Vandithavalam      CTTATTGGAGATGATCAAATTTACAATGTTATTGTTACAGCTCATGCTTTTATTATAATT
          153


AM15-Adimali            TTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATA
          222
AM14-Maneed             TTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATA
          222
AM07-Canoly             TTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATA
          198
AM05-Meenavallam        ------------------------------------------------------------
          0
AM06-Dhoni              TTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATA
          240
AM12-Kulappulli         TTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATA
          208
AM08-Mukkali            TTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATA
          224
AM09-Kalady             TTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATA
          237
AM13-Vandithavalam      TTCTTCATGGTTATACCAATTGTAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATA
          213


AM15-Adimali            TTAGCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTGCTACCC
          282
AM14-Maneed             TTGGCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCC
          282
AM07-Canoly             TTAGCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCC
          258
AM05-Meenavallam        ------------------------------------------------------ACCC
          4
AM06-Dhoni              TTAGCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCC
          300
AM12-Kulappulli         TTAGCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCC
          268
AM08-Mukkali            TTAGCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCC
          284
AM09-Kalady             TTAGCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCC
          297
AM13-Vandithavalam      TTAGCAGCCCCTGATATAGCTTTCCCTCGTATAAATAATATAAGCTTTTGATTACTACCC
          273
                                                                                ****

AM15-Adimali            CCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGA
          342
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AM14-Maneed             CCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGA
          342
AM07-Canoly             CCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGA
          318
AM05-Meenavallam        CCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGA
          64
AM06-Dhoni              CCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGA
          360
AM12-Kulappulli         CCATCATTGACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGA
          328
AM08-Mukkali            CCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGA
          344
AM09-Kalady             CCATCATTAACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGA
          357
AM13-Vandithavalam      CCATCATTGACTCTTCTTCTTGCATCCTCAATTGTTGAAAGAGGAGTTGGTACAGGATGA
          333
                        ******** **************************************************

AM15-Adimali            ACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAGCA
          402
AM14-Maneed             ACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAGCA
          402
AM07-Canoly             ACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAGCA
          378
AM05-Meenavallam        ACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAGCA
          124
AM06-Dhoni              ACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAGCA
          420
AM12-Kulappulli         ACTGTTTACCCACCTCTTTCTGCAGGTATTGCTCATGCAGGAGCTTCTGTTGATTTAGCA
          388
AM08-Mukkali            ACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAGCA
          404
AM09-Kalady             ACTGTTTATCCACCTCTTTCTGCAGGTATTGCTCATGCAGGGGCTTCTGTTGATTTAGCA
          417
AM13-Vandithavalam      ACTGTTTACCCACCTCTTTCTGCAGGTATTGCTCATGCAGGAGCTTCTGTTGATTTAGCA
          393
                        ******** ******************************** ******************

AM15-Adimali            ATTTTCAGCCTTCACCTAGCTGGGGTCTCTTCTATTCTTGGTGCTGTAAATTTTATTACT
          462
AM14-Maneed             ATTTTCAGCCTTCACCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACT
          462
AM07-Canoly             ATTTTCAGCCTTCACCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACT
          438
AM05-Meenavallam        ATTTTCAGCCTTCACCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACT
          184
AM06-Dhoni              ATTTTCAGCCTTCACCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACT
          480
AM12-Kulappulli         ATTTTTAGCCTTCATCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACT
          448
AM08-Mukkali            ATTTTCAGCCTTCATCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACT
          464
AM09-Kalady             ATTTTCAGCCTTCATCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACT
          477
AM13-Vandithavalam      ATTTTTAGCCTTCATCTAGCTGGGGTTTCTTCTATTCTTGGTGCTGTAAATTTTATTACT
          453
                        ***** ******** *********** *********************************

AM15-Adimali            ACAGTTATTAACATACGATTATCTTACATAACTCTAGACCGTATACCTTTATTTGTTTGA
          522
AM14-Maneed             ACAGTTATTAACATACGATTATCTTACATAACTCTAGACCGTATACCTTTATTTGTTTGA
          522
AM07-Canoly             ACAGTTATTAACATACGATTATCTTACATAACTCTAGACCGTATACCTTTATTTGTTTGA
          498
AM05-Meenavallam        ACAGTTATTAACATACGATTATCTTACATAACTCTAGACCGTATACCTTTATTTGTTTGA
          244
AM06-Dhoni              ACAGTTATTAACATACGATTATCTTACATAACTCTAGACCGTATACCTTTATTTGTTTGA
          540
AM12-Kulappulli         ACAGTTATTAACATACGATTATCTTATATAACTCTAGATCGTATACCTTTATTTGTTTGA
          508
AM08-Mukkali            ACAGTTATTAACATACGATTATCTTATATAACTCTAGACCGTATACCTTTATTTGTTTGA
          524
AM09-Kalady             ACAGTTATTAACATACGATTATCTTATATAACTCTAGACCGTATACCTTTATTTGTTTGA
          537
AM13-Vandithavalam      ACAGTTATTAACATACGATTATCTTATATAACTCTAGATCGTATACCTTTATTTGTTTGA
          513
                        ************************* *********** *********************
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AM15-Adimali            TCAGTTGTAATTACAGCTATCCTTCTATTATTATCTCTACCAGTTTTAGCTGGTGCTATT
          582
AM14-Maneed             TCAGTTGTAATTACAGCTATCCTTCTATTATTATCTCTACCAGTTTTAGCTGGTGCTATT
          582
AM07-Canoly             TCAGTTGTAATTACAGCTATCCTTCTATTATTATCTCTACCAGTTTTAGCTGGTGCT---
          555
AM05-Meenavallam        TCAGTTGTAATTACAGCTATCCTT-----------------------------------
          268
AM06-Dhoni              TCAGTTGTAATTACAGCTATCCTTCTATTATTATCTCTACCAGTTTT-------------
          587
AM12-Kulappulli         TCAGTTGTAATTACAGCTATCCTTCTATTATTATCTTTACCAGTTTTAGCTGGTGCTATT
          568
AM08-Mukkali            TCAGTTGTAATTACAGCTATCCTTCTATTATTATCTCTACCAGTTTTAGCTGGTGCTATT
          584
AM09-Kalady             TCAGTTGTAATTACAGCTATCCTTCTATTATTATCTCTACCAGTTTTAGCTGGTGCTATT
          597
AM13-Vandithavalam      TCAGTTGTAATTACAGCTA-----------------------------------------
          532
                        ******************

AM15-Adimali            ACTATATTATTAACAGACCGAAATCTAAATACTTCTTTCTTTGACCCTGCAGGAGGGGGA
          642
AM14-Maneed             ACTATATTATTAACAGACCGAAATCTAAATACTTCTTTCTTTGACCCTGCAGGAGGGG--
          640
AM07-Canoly             ------------------------------------------------------------
          555
AM05-Meenavallam        ------------------------------------------------------------
          268
AM06-Dhoni              ------------------------------------------------------------
          587
AM12-Kulappulli         ACTATATTATTAACAGATCGAAATCTAAATACTTCTTTCTTTGACCCTGCAGGAGGGGGG
          628
AM08-Mukkali            ACTATATTATTAACAGATCGAAATCTAAATACTTCTTT----------------------
          622
AM09-Kalady             ACTATATTATTAACAGATCGAAATCTAAATACTTCTTTCTTT------------------
          639
AM13-Vandithavalam      ------------------------------------------------------------
          532


AM15-Adimali            GA---         644
AM14-Maneed             -----         640
AM07-Canoly             -----         555
AM05-Meenavallam        -----         268
AM06-Dhoni              -----         587
AM12-Kulappulli         GACCC         633
AM08-Mukkali            -----         622
AM09-Kalady             -----         639
AM13-Vandithavalam      -----         532
```

*indicates complimentary region, -- indicates missing nucleotides

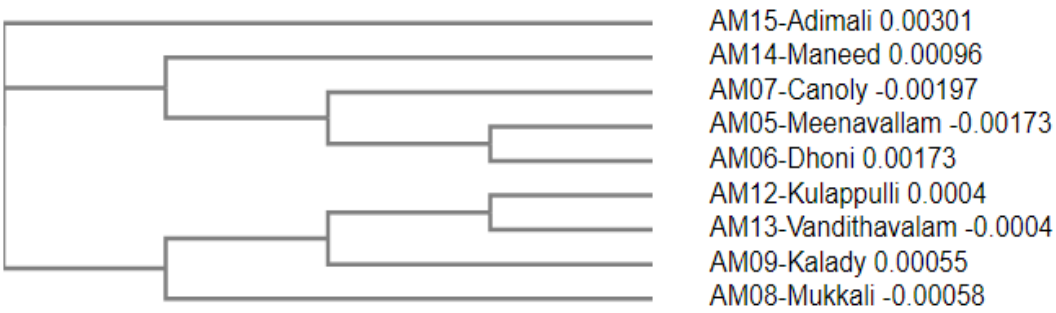


Fig. 1. Phylogenetic tree results of *Myrmeleon pseudohyalinus* populations from Clustal omega

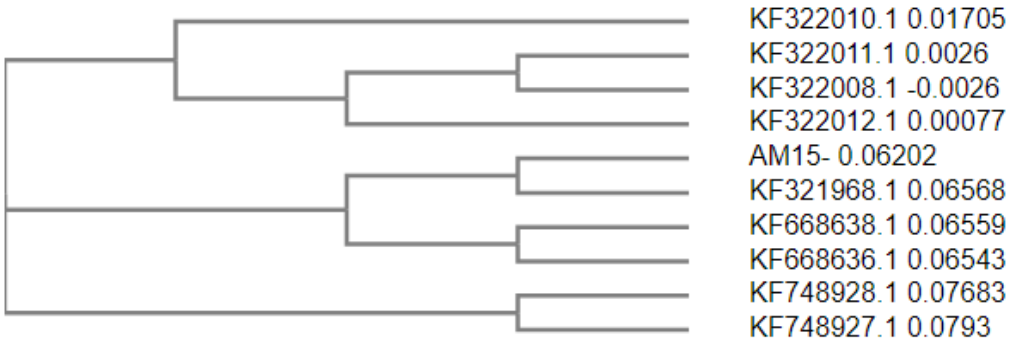


Fig. 2. Phylogenetic tree result of *M. pseudohyalinus* with related species from NCBI

2.4.3. Habitat of Antlion larvae (Genus Myrmeleon)

The four different habitats of antlion larvae Genus *Myrmeleon* classified from the study is described below

2.4.3.1. Abandoned area (9 study sites)

Here the soil vibrations were low when compared to human dwelling areas, so that they can make funnel shaped pits more cost effective and sense the arrival of even a small prey species. Here sand, soil and m-sand materials were observed as pit building media and the shaded areas were preferred by larvae. In abandoned areas, the soil texture was sand or fine sand. The mean percentage of sand, silt and clay was 89.5, 3 and 7.5 respectively. There are limited numbers of plants or trees present in these habitats because of the presence of walls and ceilings. The trees include coconut, teak etc with small canopy and mainly large trees with lesser quantity of small sized pollinators, the vegetation and antlion larval population was not that much correlated here. Also the direct light illumination was low in these areas.

2.4.3.2. Human dwelling area (27 study sites)

Here also antlion larvae preferred shaded areas but the presence of human being and other animals like goat, cattle, dogs, cats were high. But the designs of pits built in these areas were found without causing much damage by rain during monsoon. The light illumination was also less in these habitats but the soil

vibration was high. The soil texture was sand (86%) or fine sand (14%).The mean percentage of sand, silt and clay was 86, 2 and 12 respectively in these areas.

The prey capture success was high in human dwelling areas because of the presence of ants in these areas in large quantity and also they are considered as the common pest of human beings. The plants and trees are more in human dwelling areas, so that the insect pollinators and small organisms were rich in these areas.

2.4.3.3. Forest boundary area (7 study sites)

In these habitats, lowsoil vibration and light illumination were noted. Here the vegetation was somewhat high and soil and atmospheric temperatue was low.The soil texture was sand (67%) and sometimes fine sand (33%). The mean percentage of sand, silt and clay was 90, 1 and 9 respectively.

2.4.3.4. Riparian (7 study sites)

River banks were considered as riparian habitat, also found antlion larval pits under large coconut trees beside the river. The soil texture was sand (75%) and fine sand (25%). The mean percentage of sand, silt and clay was 87, 2 and 11 respectively. The wind was somewhat high in these habitats and the soil vibrations were somewhat low. The vegetation was thick in these areas so that the insect pollinators were present at an increased level.

A total of 68 study sites were visited and the antlion larvae were collected from 50 sites which include 9 abandoned areas, 27 human dwelling areas, 7 forest areas and 7 riparian areas (Table 15 and 16). The district wise distribution of genus *Myrmeleon* was plotted in Map 3 (Palakkad), Map 4 (Thrissur), Map 5 (Thiruvananthapuram), Map 6 (Wayanad), Map 7 (Malappuram), Map 8 (Kottayam), Map 9 (Kasargod), Map 10 (Pathanamthitta), Map 11 (Kannur), Map 12 (Alappuzha), Map 13 (Ernakulam), Map 14 (Idukki), and Map 15 (Kollam).

Table 15. The different habitats in which the search of antlion larvae carried out (68 study sites).

Abandoned areas	Forest areas	Riparian
Parli	Thiruvizhamkunnu	Parali Riverbank
Edathara	Bengalow kunnu	Poomala
Pezhumpara	Dhoni temple	Ezhattumugham

Murukkumpara	Soochippara	Kuruva
Prakruthigramam	Meenvallam	Thumboormuzhi
Tagore theatre	Mukkali	Vettilappara
Kanalpalam	Canoly	Meenkara
Walayar	Poabs Tea factory	Kiriyathupara
Pattikkad	Kakkayam	Ottappalam river
Sarovaram park	Thusharagiri	
Nedumbasserry		
Athani		
Sulthan Bathery		
Kadamanchira		
Human dwelling areas		
Parli Manamthody	Kinavallur	Suvarnodyanam
Wadakkancherry	Meenakshivilas	Padur
Nedupuzha	Thathamangalam	CPCRI, Chowki
Kodungallur	Thiruvalathur	Nangiarkulangara
Vellayani	Asarikkadu	Kollam Railway station
Brennan college campus	Ramakrishnappadi	Ettumanur
Thennal resort, Kattikulam	Melarankode quarters	Vadakkencherry
Nilambur Dippo	Tanur	Moyan modal school
Irrigation office	Kanimangalam	Vettikkattiri
Idimuzhikkal	Vandithavalam	Kanniyampuram
Marthoma college, Tiruvalla	Mananchira square	Kulappulli
Maneed	Adimali	

Table 16. The habitats in which the presence of antlion larvae observed (50 study sites).

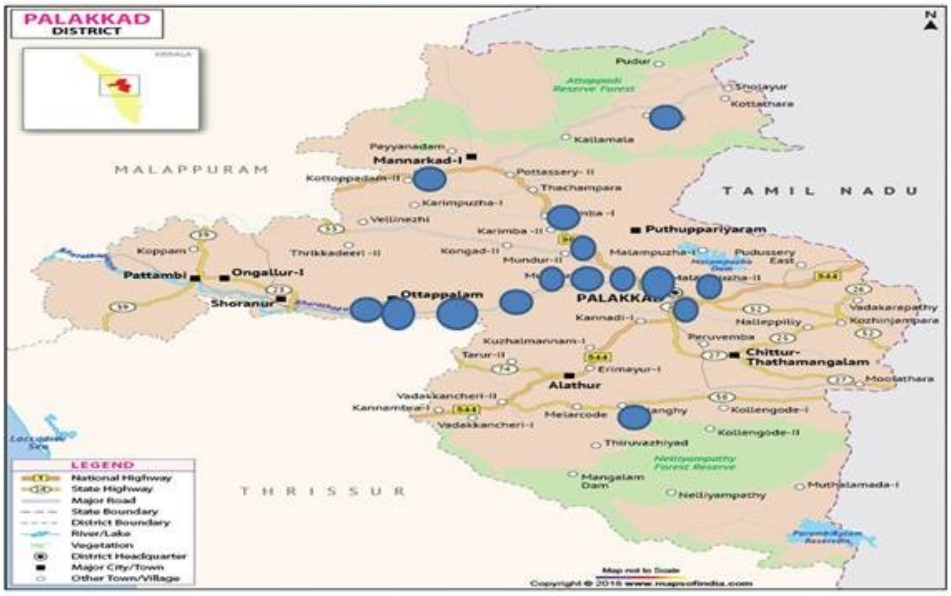
Abandoned	**Forest**	**Riparian**
Parli	Thiruvizhamkunnu	Parali Riverbank
Edathara	Bengalow kunnu	Poomala
Pezhumpara	Dhoni	Ezhattumugham

Murukkumpara	Soochippara	Kuruva
Prakruthigramam	Meenvallam	Thumboormuzhi
Tagore theatre	Mukkali	Vettilappara
Pattikkad	Canoly	Ottappalam river
Sulthan Bathery		
Kadamanchira		
Human Dwellings		
Parli Manamthody	Kinavallur	CPCRI, Chowki
Wadakkancherry	Thiruvalathur	Nangiarkulangara
Nedupuzha	Asarikkadu	Kollam Railway station
Kodungallur	Ramakrishnappadi	Ettumanur
Vellayani	Melarankode quarters	Moyan modal school
Brennan college campus	Tanur	Vettikkattiri
Nilambur Dippo	Idimuzhikkal	Kanniyampuram
Thennal resort, Kattikulam	Adimali	Irrigation office
Marthoma college, Tiruvalla	Maneed	Kulappulli

2.4.4. Morphometric Analysis-Larvae

M. pseudohyalinus larvae were collected from seven study areas (Table 10) coming under four districts of Kerala namely Palakkad, Malappuram, Ernakulam and Idukki. Morphometric measurements of the second and third instar of the species were taken, the LBL, LBW, LHL, LHW and ML (Table 17 and 18) were measured and compared with the habitats. Collections were taken from forest boundaries (Meenvallam, Dhoni, Canoly, and Mukkali) and human dwelling areas (Kulappulli, Maneed and Adimali). The specimens were brought to laboratory for rearing purpose (Plate 13) and the photographs were taken and given in Plate 18.

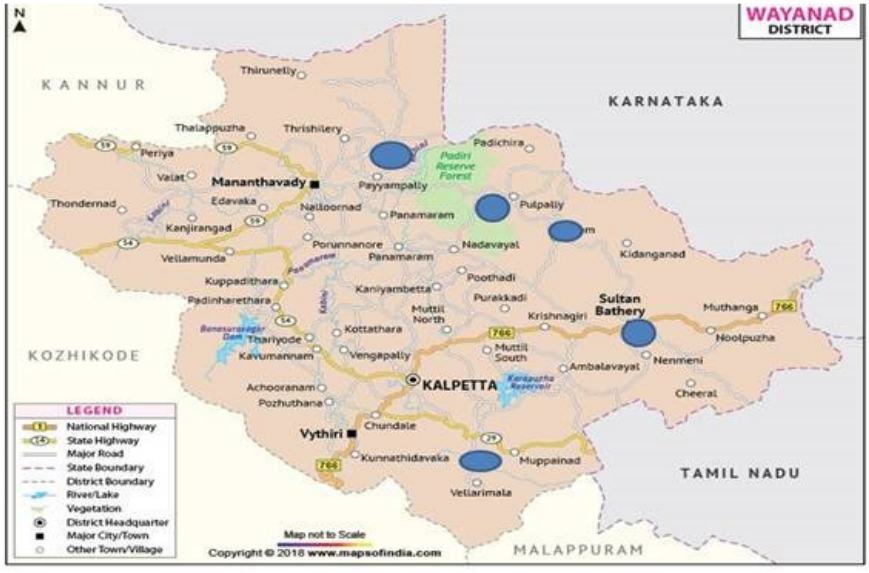
The second instar larvae with highest value of mean LBL were collected from Canoly (MLM) and lowest values of mean LBL from Dhoni (PKD). The values of mean LBL ranged from 0.5 to 0.8 cm. The mean LBL from seven different sites showed slight difference as indicated by the standard deviation of 0.098. Mean LBW, LHL, LHW and ML also showed similar trend with the values collected



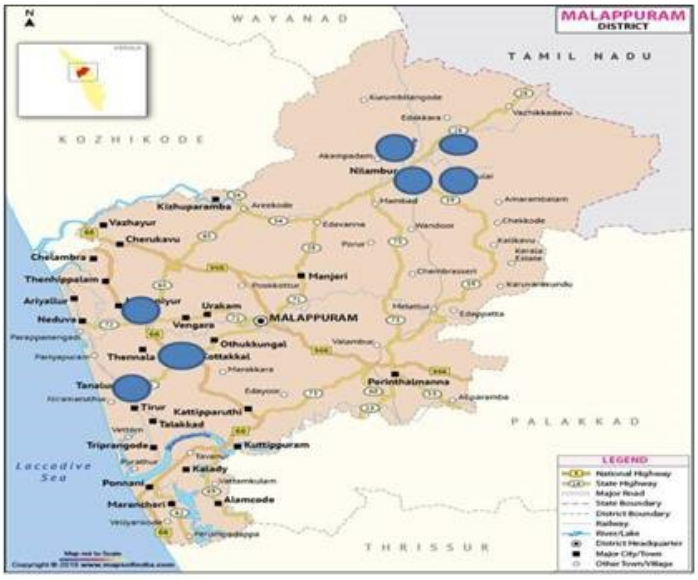
Map 3- Distribution of Genus *Myrmeleon* in Palakkad Dstrict

Map 4- Distribution of Genus M*yrmeleon* in Thrissur District

Map 5- Distribution of Genus *Myrmeleon* in Thiruvananthapuram District



Map6- Distribution of Genus *Myrmeleon* in Wayanad District



Map 7- Distribution of Genus *Myrmeleon* in Malappuram District

Map 8- Distribution of Genus *Myrmeleon* in Kottayam District

Map 9- Distribution of Genus *Myrmeleon* in Kasargod District

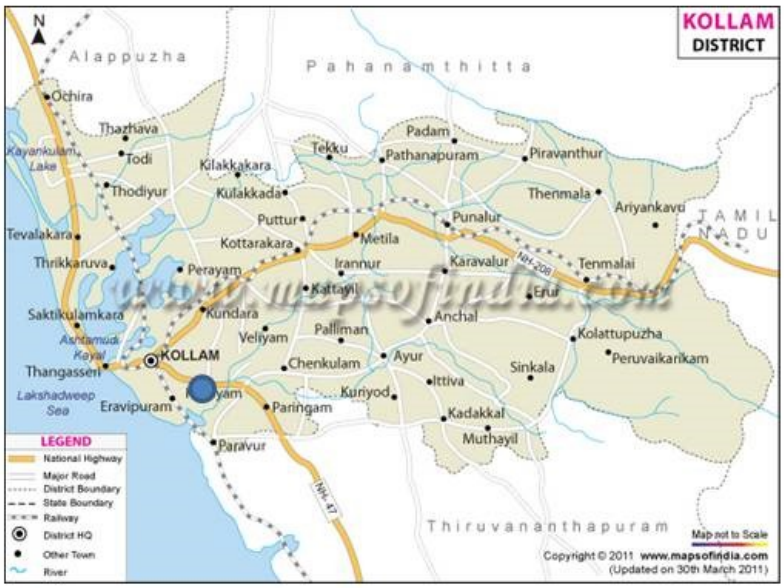
Map 10- Distribution of Genus *Myrmeleon* in Pathanamthitta District

Map 11- Distribution of Genus *Myrmeleon* in Kannur District

Map 12- Distribution of Genus *Myrmeleon* in Alappuzha District

Map13- Distribution of Genus *Myrmeleon* in Ernakulam District

Map14- Distribution of Genus *Myrmeleon* in Idukki District



Map15- Distribution of Genus *Myrmeleon* in Kollam District

from different sites having almost similar.Standard deviation for LBW, LHL, LHW and ML were 0.038, 0.079, 0.053 and 0.053 respectively. Therefore it may be assumed that *M. pseudohyalinus* second instar larvae from study area would follow the above morphometric measurement range (Fig.3).

The third instar larvae with highest value of mean LBL were collected from Meenvallam (PKD) and Canoly (MLM) and lowest values from Mukkali (PKD), Maneed (ERN) and Adimali (IDKI). The values of LBL ranged from 0.8 to 1 cm. The LBL from seven different sites showed very little difference as indicated in the standard deviation of 0.089. Mean LBW, LHL, LHW and ML also showed similar trend with the values collected from different sites having almost similar. Standard deviation for LBW, LHL, LHW and ML were 0.049, 0.053, 0 and 0.053 respectively. Therefore it may be assumed that *M. pseudohyalinus* third instar larvae from study area would follow the above morphometric measurement range (Fig. 4).

The five body measurements of larvae of *Myrmeleon sp.* and two measurements of cocoon were compared by using Pearson correlation for parametric variables at 5% significant level (Appendix 1). Here, positive correlation were found between body width and body length , body length and head length, body width and head length, body width and head width (Fig. 7).

The shape and size of a body and its body parts both in absolute and relative term are a function of its ecological role. The shape and size determines how efficiently an organism can perform different activities both general and specialized. Correlation between certain body parts or traits would indicate a symmetry between them which in turn would determine its efficacy in its chosen habit and habitat.

Since this is a preliminary study, there is no data available on these species regarding body shape, size and relative size of body parts. The objective in presenting the data here is to provide a baseline data from where other studies can take off.

Plate 13

Rearing of Antlion larvae

Adult spreading

collection

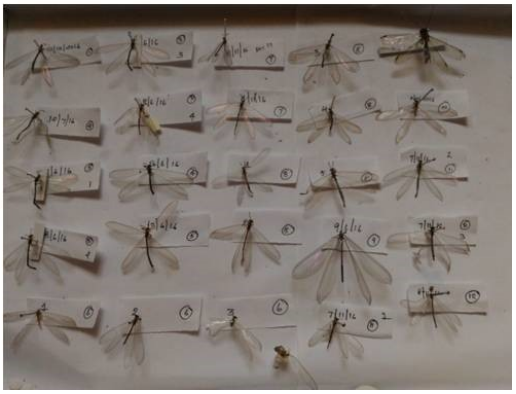
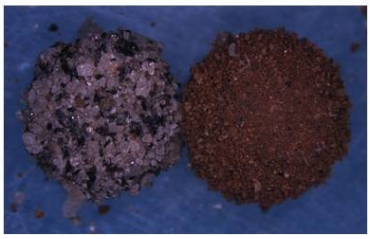


Plate 14

Larvae

Adult



Types of cocoons

Cocoon with exuviae

Cocoon with out exuviae

Exuviae

Plate 15

Collected soil samples

Microscope

Plate 16

Weighing of Antlion Cocoon

Slide making

Correlation

	PDE	*PDI*	*LHL*	*LHW*	*LBL*	*LBW*
PDE	1					
PDI	-0.08251	1				
LHL	-0.22056	0.216193	1			
LHW	-0.16066	0.355969	0.681227	1		
LBL	0.021572	0.397212	0.371056	0.596104	1	
LBW	-0.1565	0.587012	0.58401	0.513509	0.518773	1

Fig. 5. Correlation results showing relationships between the different parameters of pit size and larval size. (Krishnan & Kakkassery, 2016)

Relationship between pit size and larval size were studied (Fig. 5) and from the correlation results it was noted that, there is a negative correlation between larval head length and pit depth (Fig. 7). That is, the pit depth increased with the decrease in larval head length. So smaller the head length of antlion larvae, bigger the depth of pits. Also, there is a high positive correlation between larval body width and pit diameter (Fig. 6). That is the pit diameter increases with the increase in larval body width (Plate 17). There is no relationship between the larval body length and pit depth. The larval body width may help the larvae to make the pits with larger diameter. From this study, it is clear that other parameters of pit do not have any relationship with the larvae dwelling inside. Also there is a high positive correlation between pit depth and diameter (fig. 8). The pit depth increases with the increase in diameter.

The high positive correlation results of larval body width and pit diameter were used for the instar determination study. Here the pit diameter classified into three classes according to their values, that are 1.2-2.2, 2.2-3.2, 3.2-4.2, from these classes the mean larval body width in each diameter was calculated by taking its average (Table 17). The result is furnished below.

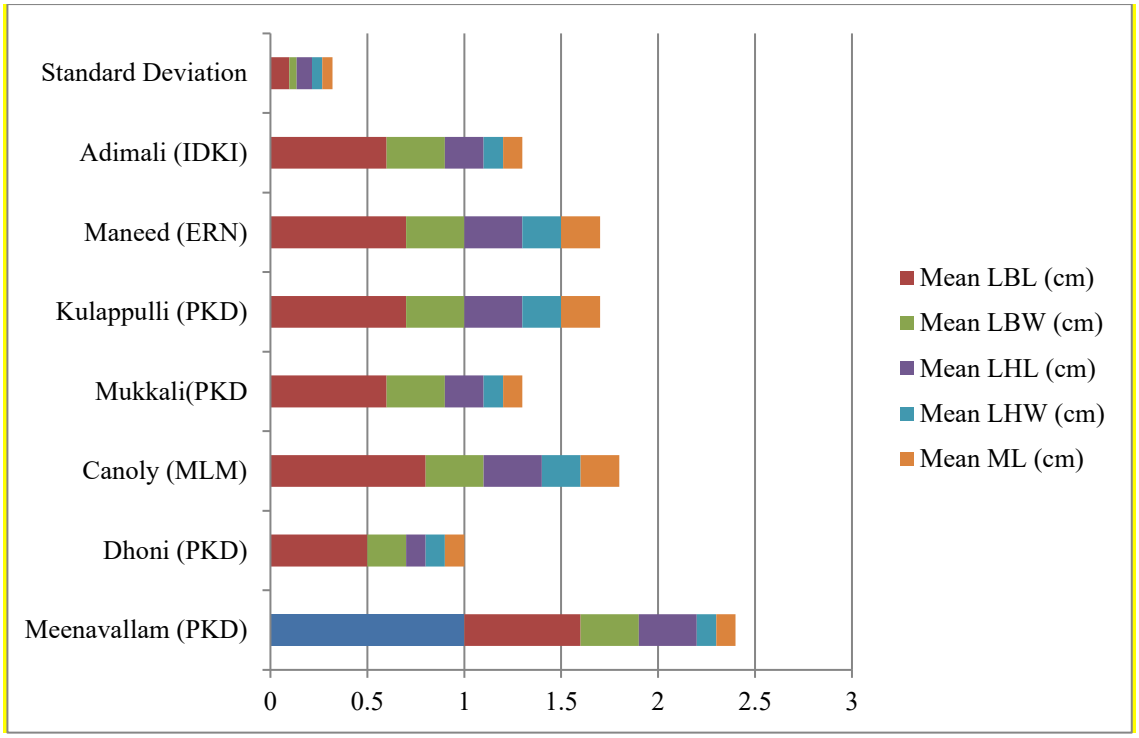


Fig.3. Comparison of body measurements of second instar *Myrmeleon pseudohyalinus* larvae in different study areas of Kerala

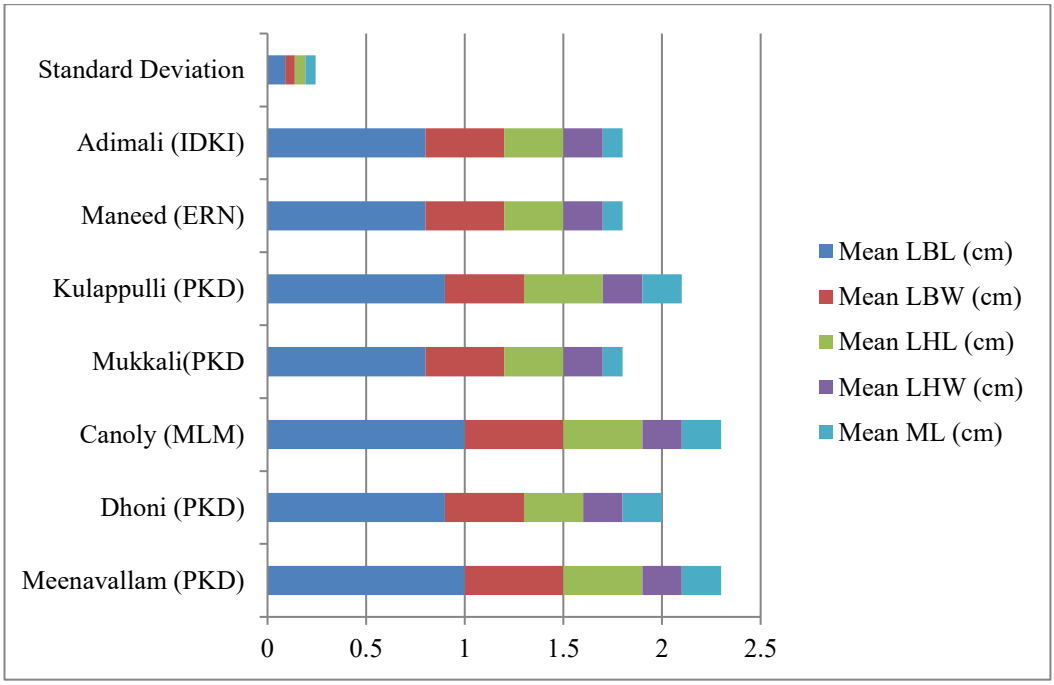


Fig.4. Comparison of body measurements of third instar *Myrmeleon pseudohyalinus* larvae in different study areas of Kerala

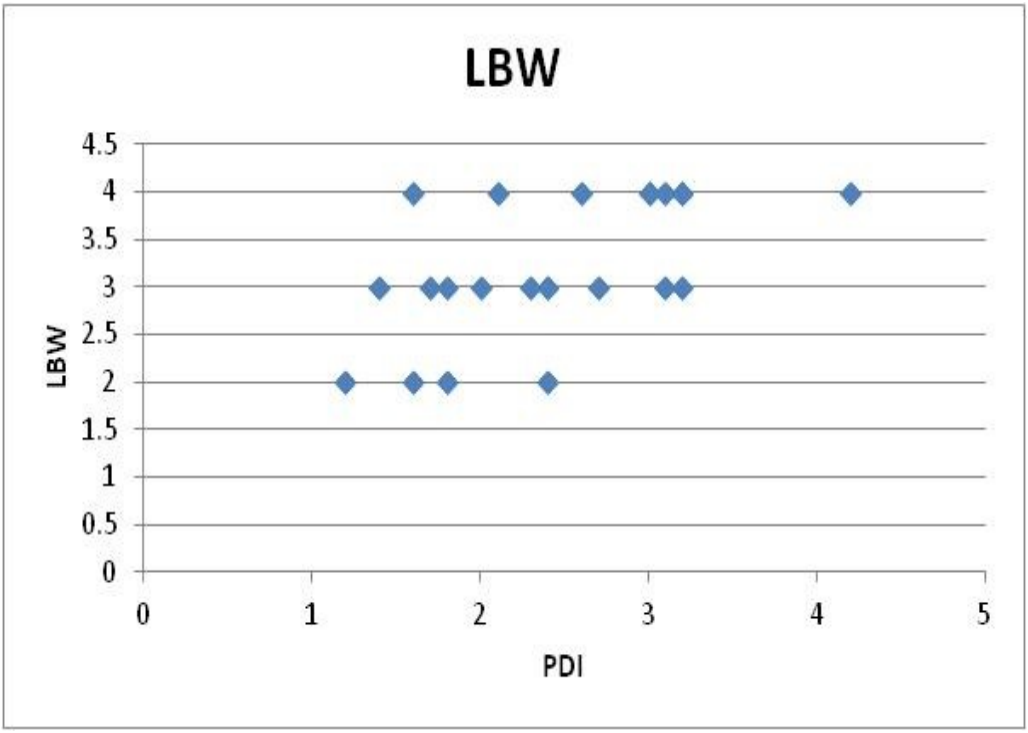


Fig. 6. Positive correlation between larval body width and pit diameter.

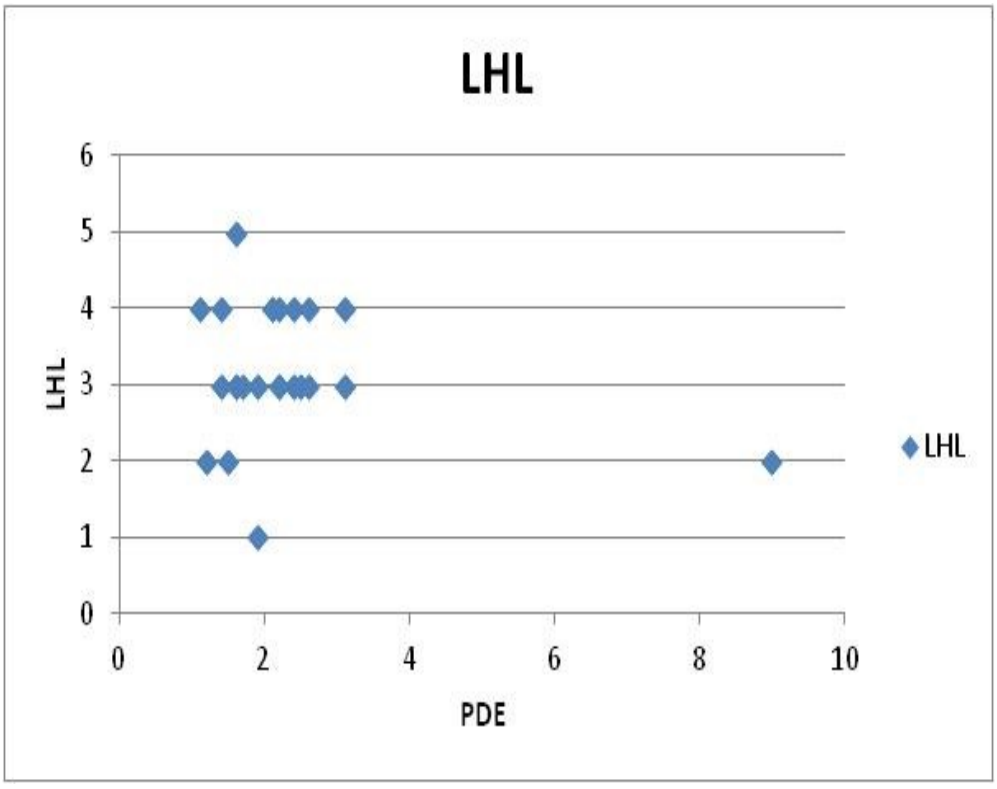


Fig.7. Negative correlation between larval head length and pit depth

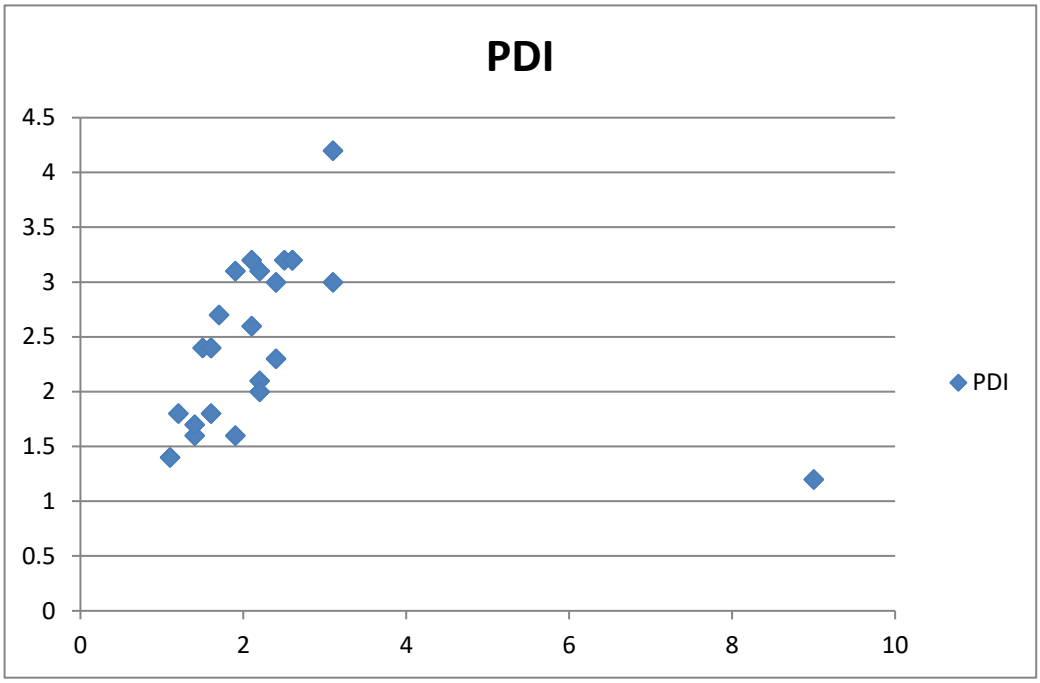


Fig. 8. showing the positive correlation between pit depth and diameter

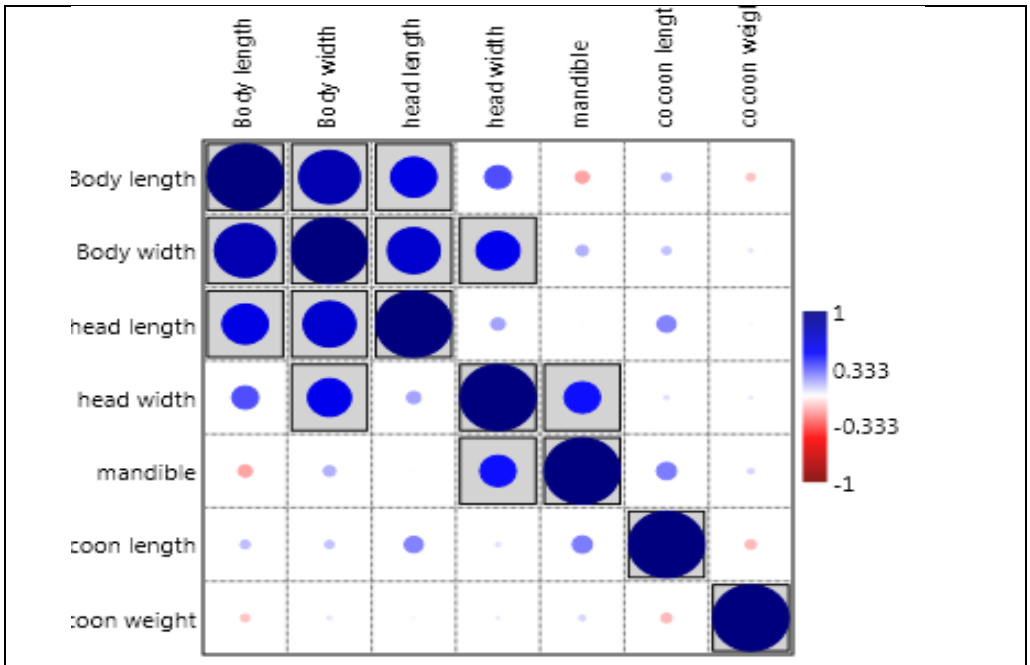
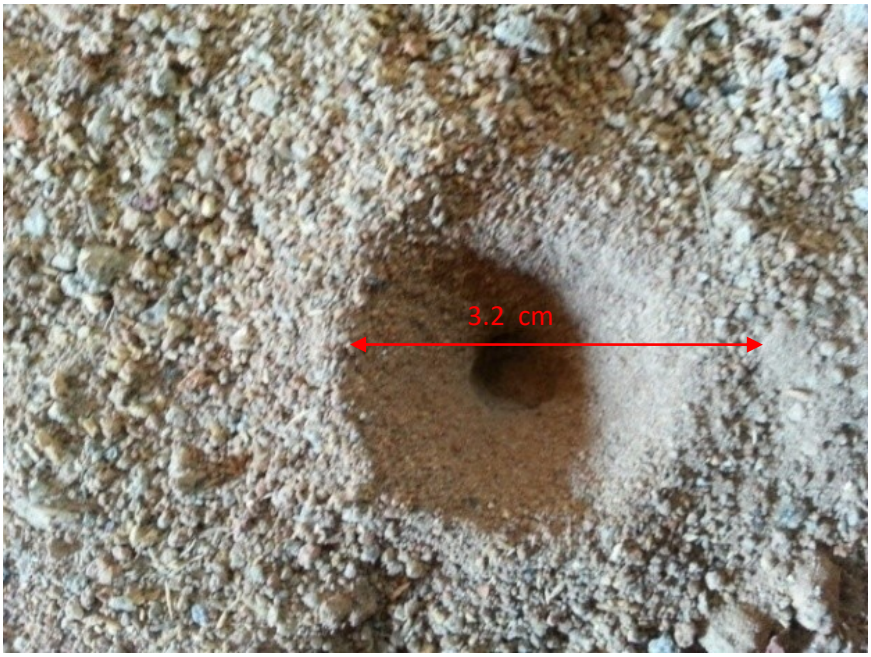


Fig. 9. Correlation plot of Genus Myrmeleon (larvae and cocoon)

Plate 17



The correlation between pit diameter and larval body width.

Plate 18

Dorsal View

Ventral View

Antennae

Compound eye

Abdomen

Abdominal tip

Table 17. The expected larval body width as per diameter of pits

PDI (cm)	LBW (number)	Mean LBW (mm)
1.2-2.2	18	2.88
2.2-3.2	20	3.35
3.2-4.2	10	3.80

From the result, we can predict the larval size of pit- dwelling larvae by measuring its diameter. It is known that antlions have three larval instars during its life cycle. Here the average size of each larva in each instar is determined. The average body width of first instar larvae is approximately 2.88 mm seen in the pit with diameter ranging from 1.2-2.2 cm. The average body width of second and third intar larvae is approximately 3.35 and 3.80mm respectively which is seen in pit diameter ranging from 2.2-3.2and 3.2-4.2 cm. Simply it can be determined that, the pits with diameters ranging from 1.2-2.2, 2.2-3.2, 3.2-4.2 cm have the larvae with body width 2.9, 3.4, 3.8 mm respectively.

Both *M. pseudohyalinus* and *M. hyalinus,* the size of all body parts showed significant positive correlation with each other (Appendix 2 and 3) (Fig. 10 and 11). Oneway Anova was performed to compare means of different body part measurements of different species. It was found that there was no significant difference in the mean LBL and mean LBW (Appendix 4 and 5) of the *M. pseudohyalinus* and *M.hyalinus* (F=0.1739; df 2, 69 at 5%). Thus it was concluded that the larval body length and larval body width were not enough to distiguish between the larvae of the two species.There was a significant difference in the mean LHL (F=3.645, df 2, 69) (Appendix 6), mean LHW (F=17.7, df 2,69) (Appendix 7) and ML (F=6.694, df 2,69) (Appendix 8) at 5% significance. This indicate that the major differentiating character between the larvae of the two species are LHL, LHW and ML.The *Myrmeleon hyalinus* has longer head (0.27 cm), wider head (0.17 cm) and longer mandible (0.16 cm) than *M. Pseudohyalinus*

2.4.5. Morphometric Analysis-Cocoon

A total of 75 cocoons of the genus *Myrmeleon* were collected from five districts of Kerala and the district wise data are given in Table 18. The photographs were taken and given in Plate 19.

Table 18. District wise collection data of cocoon coming under genus *Myrmeleon*

Sl No.	District	Number of cocoon
1	Palakkad	54
2	Thrissur	14
3	Wayanad	1
4	Thiruvananthapuram	4
5	Kannur	2
	Total	75

Dry soil, sand, m sand and brick dust were used by antlion larvae to make their cocoon for pupation. The smallest cocoons were collected from Wayanad district and largest cocoons were from Kannur district. The type of materials which forms the cocoon of antlion larvae was sand in Wayanad and dry soil in Kannur. The detailed description of circumference, diameter and weight of cocoon were given in Fig.12 and Fig.13.

2.4.6. Morphometric Analysis- Adult

The adult of genus M*yrmeleon* was small in size when compared to *Palparinae*, and *Distoleon* species*,* The morphometric measurements were done using a scale and it was given in Fig.14.

2.4.7. Physical Parameters

The average temperature was high in human dwelling areas and low in river banks. The larvae prefer high temperature, and thus show preference for human dwelling areas with shades. The soil and atmospheric temperature, humidity, dewpoint, pressure, uv index, visibility, and wind were measured in the study areas. The average soil temperature was 32^{o}C and the average atmospheric temperature was 30.1^{o}C. The maximum and minimum atmospheric temperatures were 39^{o}C and

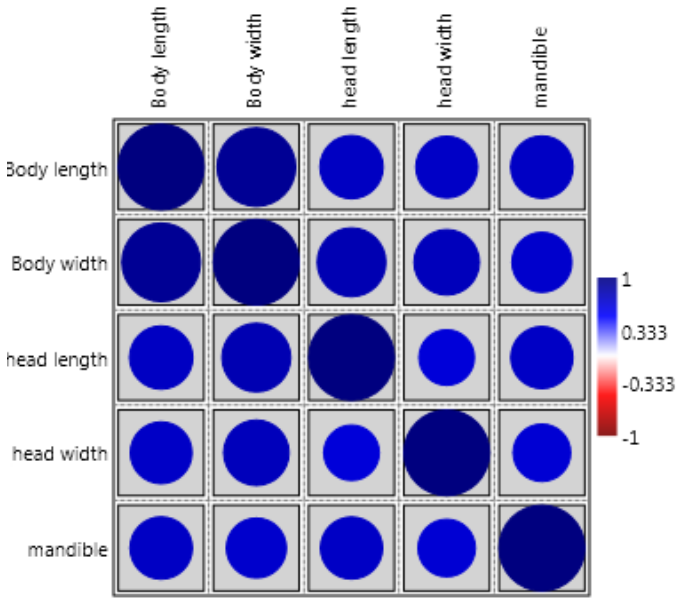


Fig. 10. Correlation plot of larval morphometry of *M. pseudohyalinus*

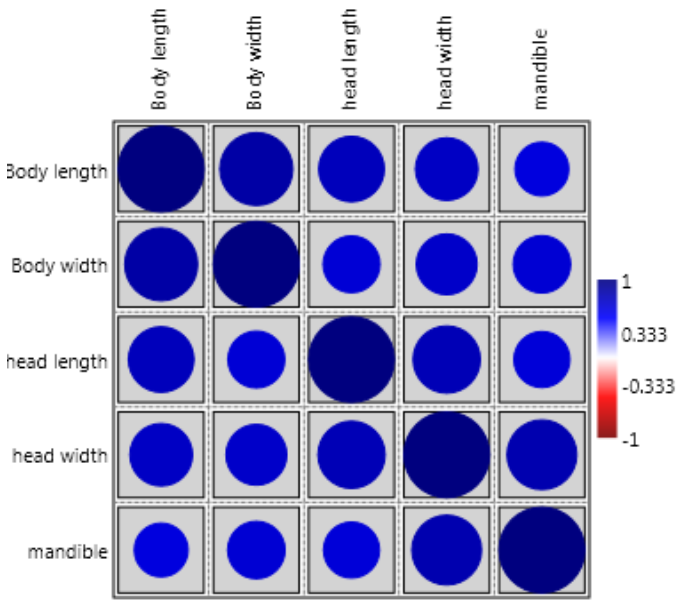


Fig. 11. Correlation Plot of larval morphometry of *M. hyalinus*

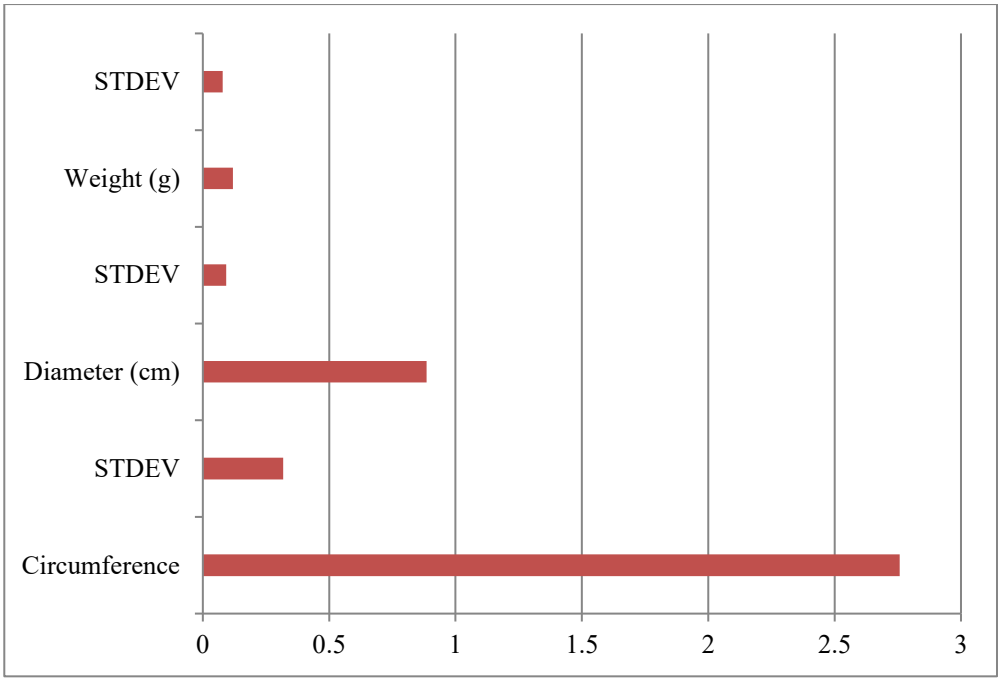


Fig.12. The average circumference, diameter and weight of cocoon collected from Kerala

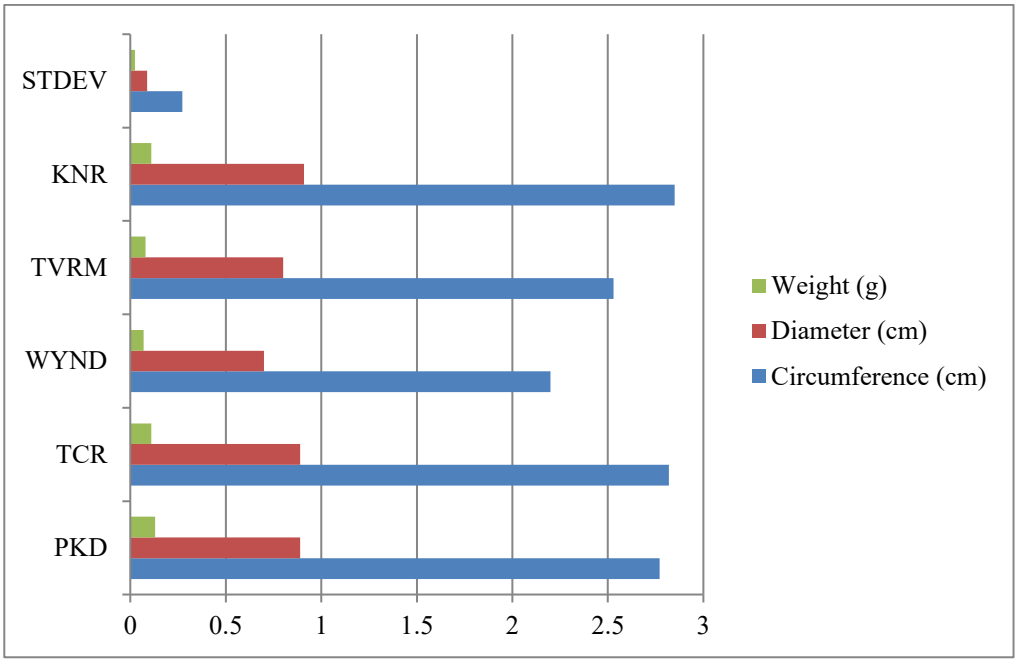
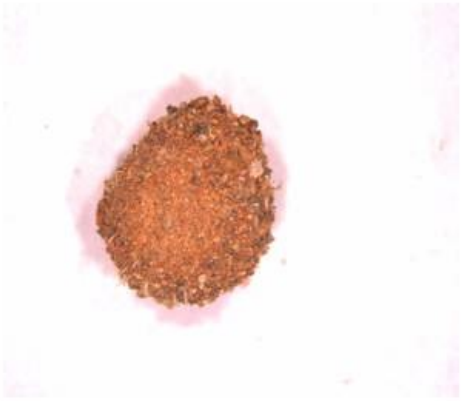


Fig.13. The average circumference, diameter and weight of cocoon collected from Different District of Kerala

Plate- 19



Cocoon in dry soil

Cocoon in dry sand

Cocoon with exuviae

Hole in cocoon

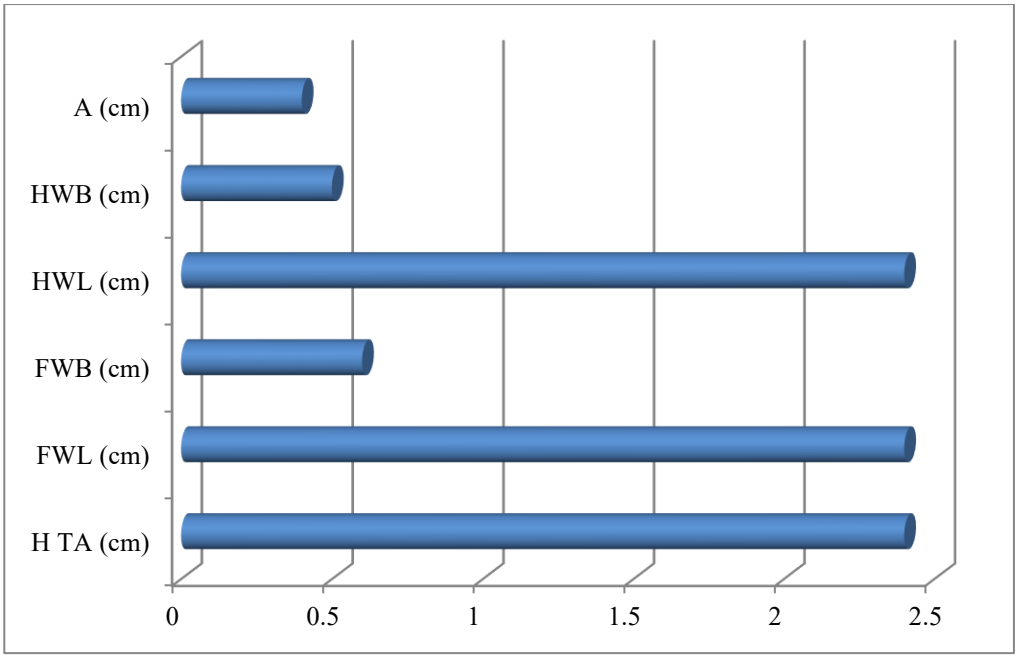


Fig.14. Average morphometric measurements of *Myrmeleon pseudohyalinus*

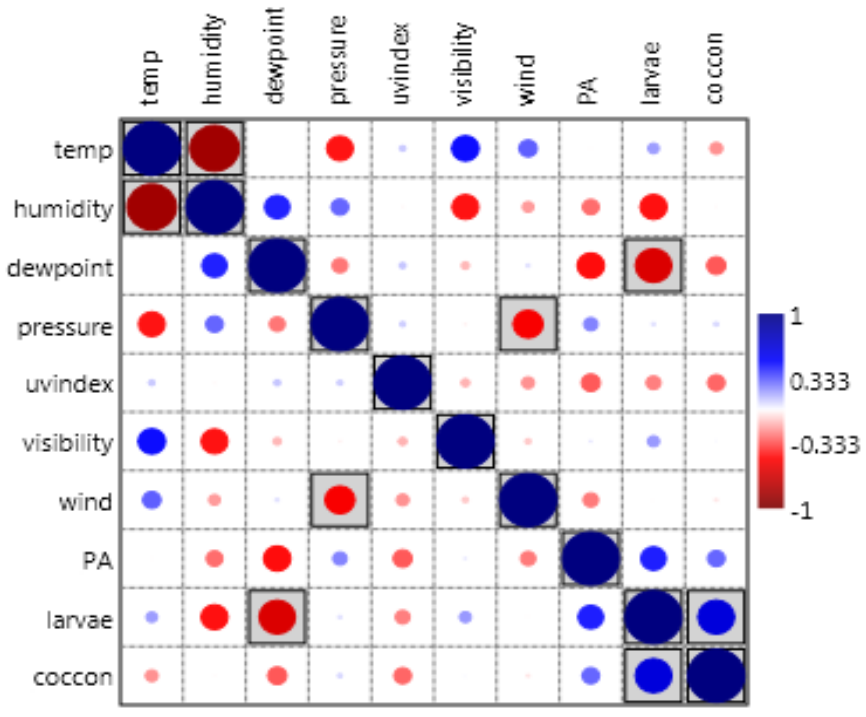


Fig.15. Correlation plot for different abiotic parameters

21ºC respectively.The maximum and minimum humidity observed were 90% and 20 %. The average humidity was 64.8%. The dew point ranges from 11ºC to 26ºC and the average was 22.3ºC.

The larvae of genus *Myrmeleon* showed a significant negative correlation to dew point and a non significant negative correlation to humidity and uv index (Fig. 15). Non significant positive correlation to temperature, visibility was also observed. The detailed comparisons of different abiotic factors present in four habitats were given in Table 19.

Table 19. Comparison of different abiotic factors observed in four habitats

Parameters	Abandoned area	Forest	Riparian	Human dwelling
Mean temperature (ºC)	31.29	30.42	29.8	30.92
Min temperature	27	20	22	22
Max temperature	36	37	36	39
Mean humidity (%)	57.25	65.17	59	59.92
Min humidity	46	42	45	20
Max humidity	65	90	81	82
Mean dewpoint (ºC)	20	23	20.8	21.2
Min dewpoint	19	19	19	11
Max dewpoint	22	25	22	24
Mean pressure (milli bars)	1010.33	1009.17	1011.8	1010.5
Min pressure	1010	1008	1009	1007
Max presure	1011	1013	1017	1008
Mean uv index	6	5.67	6.6	4.66
Min uv index	0	1	1	1
Max uv index	6	11	11	11
Mean visibility	12.33	10	11.2	12.7
Min visibility	11	3	5	8

Max visibility	13	13	16	14
Mean wind speed (km/hr)	9	10.66	10.25	10.36
Min wind speed	8	3	5	5
Max wind speed	10	13	16	14

2.4.8. Soil Texture Analysis

A total of 27 soil samples were collected from study sites in which the presence of antlion larvae was observed. It includes the study areas coming under seven districts of Kerala namely Palakkad, Thrissur, Wayanad, Malappuram, Thiruvanathapuram, Kannur and Pathanamthitta. The twenty seven soil samples were analysed for understanding the texture of substrate of these twenty one soil samples were of the texture class sand and six soil samples were of the texture class fine sand (77.8% of soil samples were in texture class sand and 22.2% was fine sand).

Ten soil samples were collected and analysed the texture by examining the percentage of the soil components (sand, silt and clay) in Palakkad District. Seven soil samples are coming under the texture class sand and three samples coming under fine sand (Fig. 16). The soil samples collected from the study area Parali, Thiruvizhamkunnam, Parli river bank were fine sand in texture and that of Ottappalam, Manamthody, Moyan school, Kinavallur, Edathara, Dhoni, and Pezhumpara were sand in texture. Ottappalam, Kinavallur and Edathara were the study sites in which the absence of silt component noted.

Seven soil samples were tested from Thrissur district (Fig. 17) in which three samples were coming under texture class fine sand and four samples were texture class sand. The soil samples collected from Asarikkadu, Wadakkanchery and Nedupuzha were fine sand in texture and Thumburmuzhi, Vallathol Nagar, Kodungallur, Poomala and Ezhattumugham were sand in texture. Kuruva and Kattikulam of Wayanad District (Fig. 18), Benglakunnu, Nilambur Dippo, Nilambur Irrigation office and Idimuzhikkal of Malappuram District (Fig. 18) were tested and the result shows the sand texture in that place. The samples collected from Vellayani of Thiruvanathapuram district (Fig. 19), Brennan college

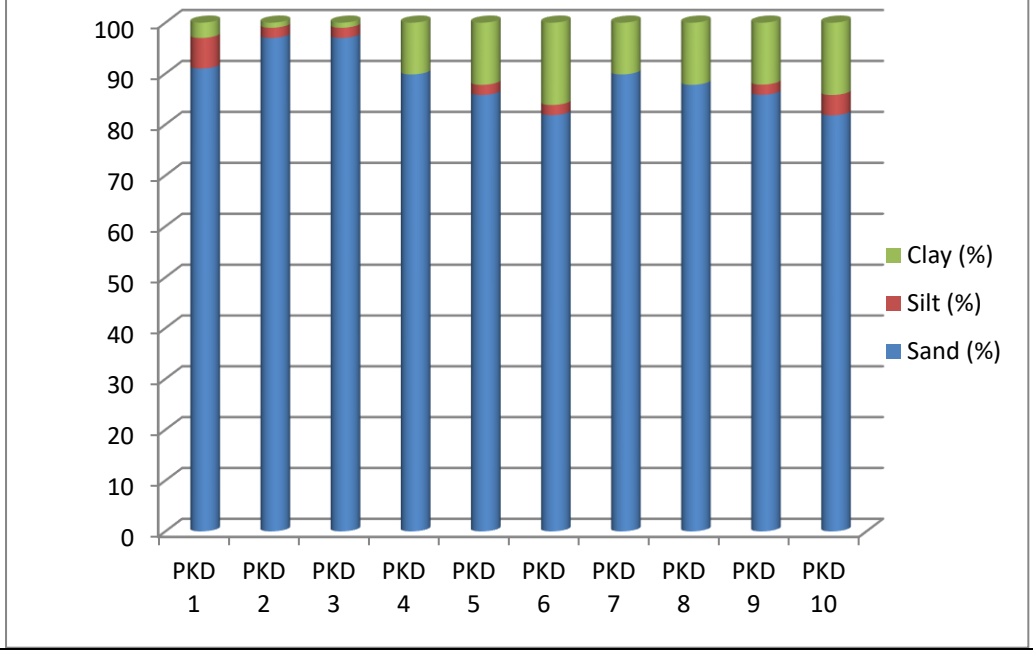


Fig. 16. The sand, silt and clay percentage of soil samples in Palakkad District PKD1=AA,PKD2=FB, PKD3=RA, PKD4=RB, PKD5=HDA, PKD6=HDA, PKD7=HDA,PKD8=AA, PKD9=FB, PKD10=AA

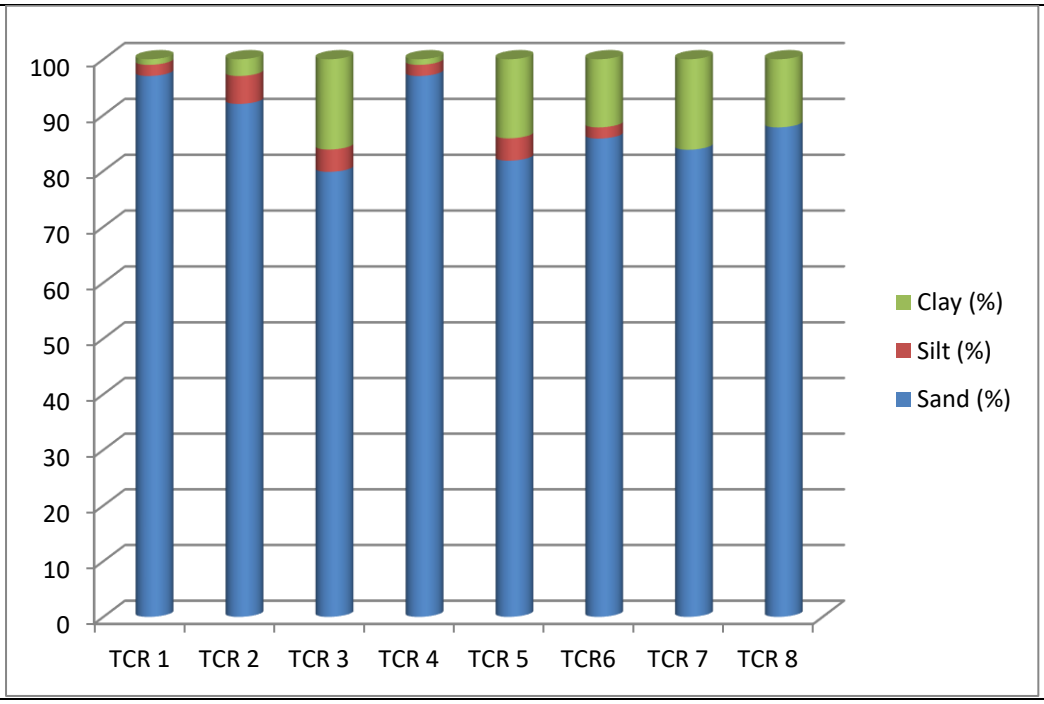


Fig 17. The sand, silt and clay percentage of soil samples in Thrissur District TCR1= HDA, TCR2=HDA, TCR3=RB, TCR4=HDA, TCR5=HDA, TCR6=HDA, TCR7=RB, TCR8=RB

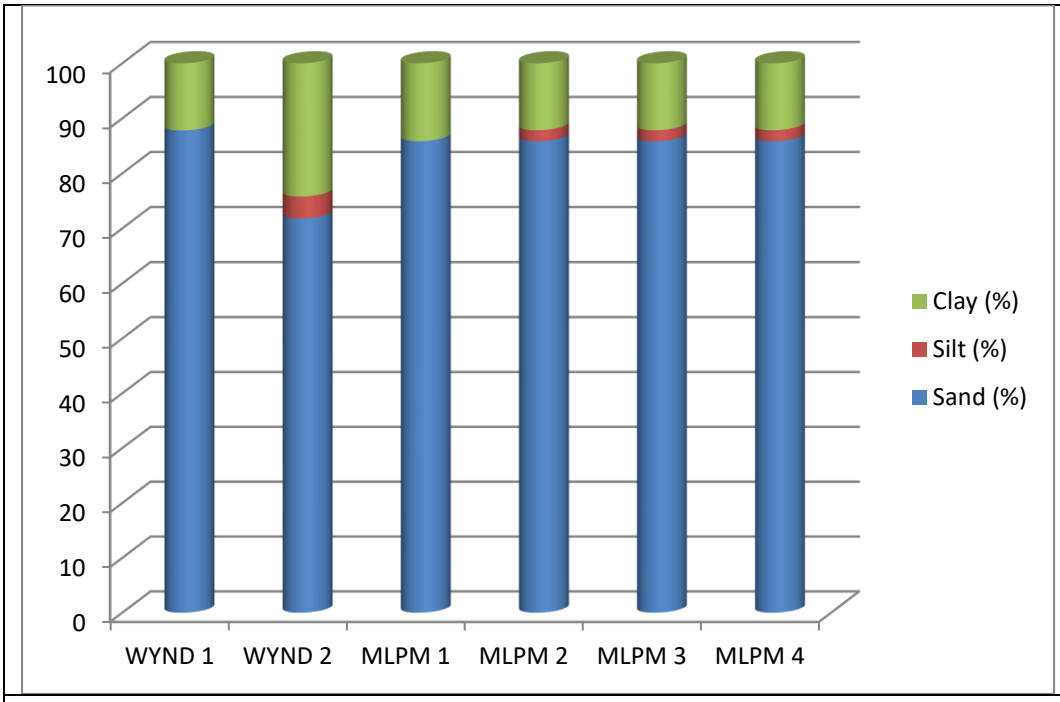


Fig. 18. The sand, silt and clay percentage of soil samples in Wayanad and Malappuram Districts. WYND1=RB, WYND2=HDA, MLPM1=FB, MLPM2=HDA, MLPM3=HDA, MLPM4=HDA

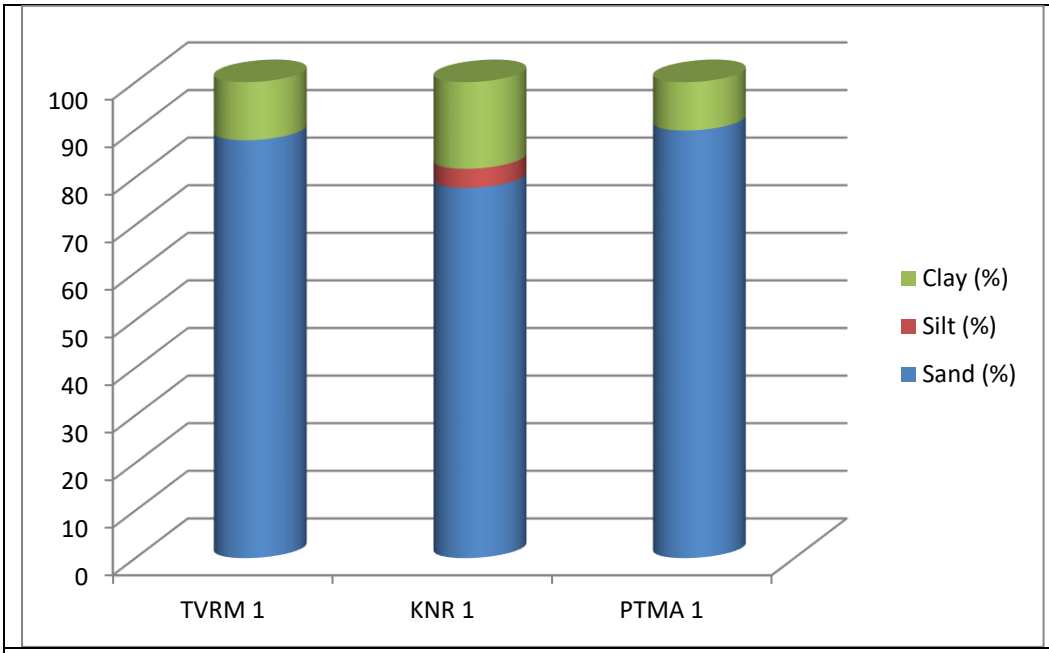


Fig. 19. Sand, silt and clay percentage of soil samples in Thiruvananthapuram, Kannur and Pathanamthitta Districts
TVRM1=HDA, KNR1=HDA, PTMA1=HDA

campus of Kannur District (Fig. 19), Tiruvalla of Pathanamthitta District (Fig. 19) were sand in texture.

In the case of Palakkad District the minimum and maximum sand percentage is noted from the soil samples collected from Pezhumpara & Moyan modal school area and Thiruvizhamkunnu & Parli river bank respectively. Pezhumpara is an abandoned area and Moyan modal school is a human dwelling area. Thiruvizhzmkunnu and Parli river banks are coming under forest and riparian respectively. In the case of Thrissur, the sand percentage was minimum in the soil collected from Thumboormuzhy and the sand percentage was maximum in the soil collected from Murikkumpara and Nedupuzha. In case of Malappuram district all the four samples were observed similar percentage of sand particle.The detailed sand, silt and clay content present in four habitats were given in Table 20 and Appendix 9.

Table 20. The sand, silt and clay content of four habitats of genus *Myrmeleon*

Sl No.	Habitat	Min sand %	Max sand %	Min silt %	Max silt %	Min clay %	Max clay %
1	**Abandoned area**	82	97	0	6	1	14
2	**Human dwelling**	72	97	0	5	1	24
3	**Riparian**	80	97	0	4	1	16
4	**Forest areas**	86	97	0	2	1	14

Number of individuals are significantly positively correlated with silt (Pearson's correlation, 5% significance) in sandy soil (Fig. 20). Soil samples collected from seven districts (total of 27 sites) indicated that sand was the predominant content, 80% of every soil sample was sand, and there was slight variation in the silt and clay content.

2.4.9. Soil Chemical Analysis

PCA for all 10 chemical parameters of soil where antlion larvae sampled and the number of individuals present were done. The soil chemical parameters are PH, EC, OC, N, P, K, Ca, Mg, S, Cl. The Mg, OC, EC, and P^H were influenced the number of individuals in a positive manner. But, Ca and K show a negative correlation with the number of individuals. The Eigen values and map were given in Table 21 and Fig. 21 respectively.

Table 21. Eigen values

PC	Eigenvalue	% variance
1	2.26E+06	97.286
2	56452.5	2.4302
3	5133.78	0.221
4	1137.15	0.048953
5	232.276	0.009999
6	75.0003	0.003229
7	6.51953	0.000281
8	2.93664	0.000126
9	0.0668348	2.88E-06
10	0.00245453	1.06E-07

2.4.10. Seasonal Adaptability and Habitat Choice

In the first, second and third experiment, the larvae made its first pit at an average of 23, 32 and 57 days respectively. In this experiment it is inferred that, in the rainy conditions they remain buried deep in the soil and waited for the right time to make its pits by analysing the soil temperature. This may also be considered as a seasonal adaptation of the species. The time taken for pit rebuilding were given in Table 22 and the pit building behaviour in natural and laboratory conditions were compared in Table 23.

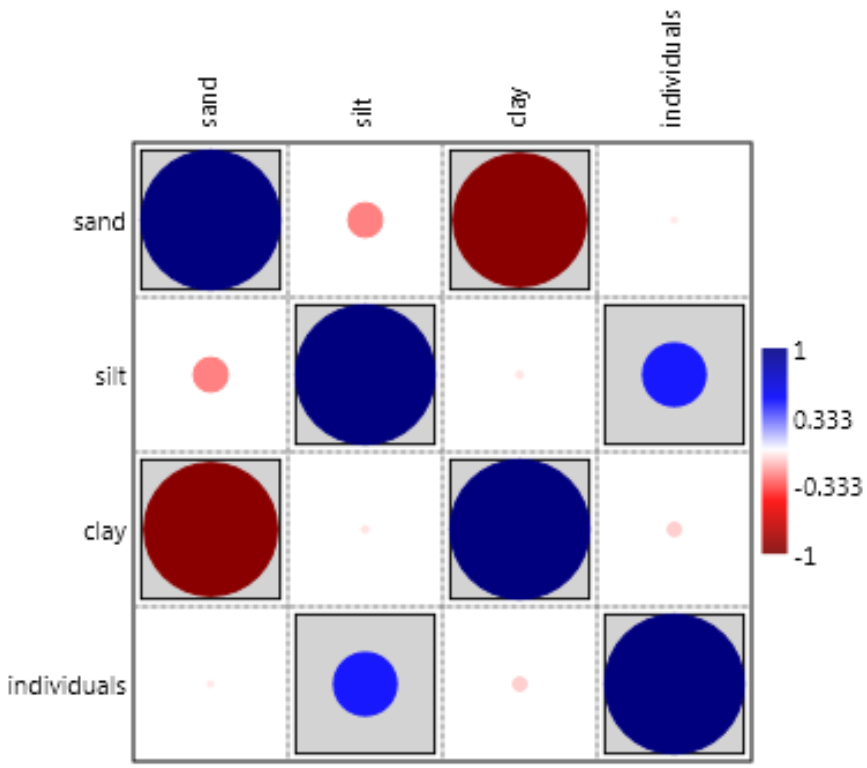


Fig. 20. Correlation plot showing the relation between soil characters and Myrmeleon larvae.

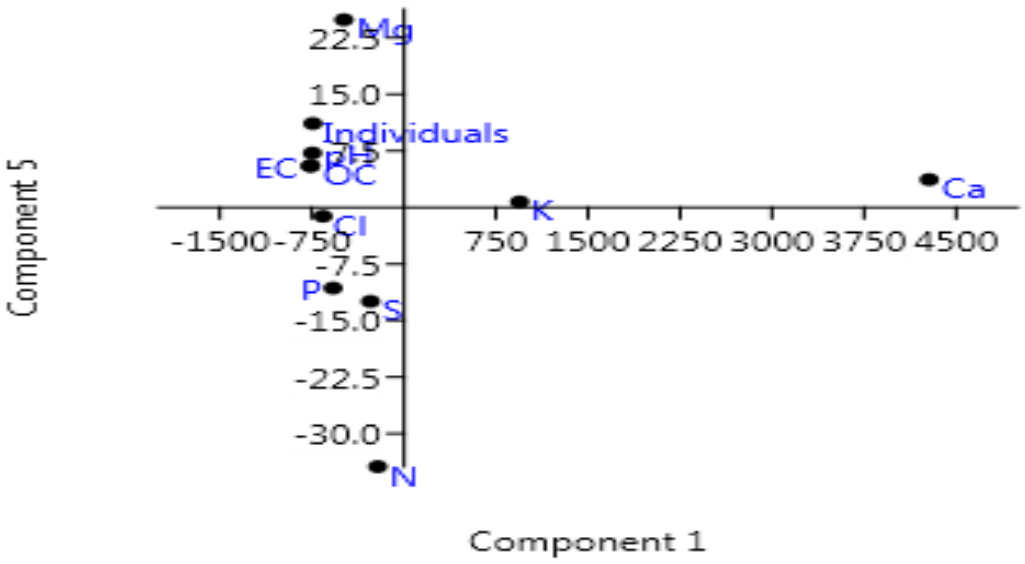


Fig. 21. PCA map showing negative correlation between K & Ca and number of individuals.

Table 22. Time taken for pit rebuilding and average temperature after the water spray

Experiment	Average time taken for pit rebuilding	Mean Temperature
Two spray	23 days	28-32^{o}C
Four spray	32 days	28-32^{o}C
Six spray	57 days	28-32^{o}C

Table 23. Comparison of pit building behaviour in natural and laboratory conditions

	Natural condition	**Laboratory condition**
Pit building in dry soil	Within 24 hours	Within 24 hours
Pit rebuilding in rainy condition	Up to 84 days	Up to 57 days
Pit depth	Same	Same
Pit diameter	Same	Same
Temperature of soil	28-32	28-32
Behaviour of larvae	In rainy season they move deep in to the soil and wait for the favourable condition (dry soil/soil temperature) to make its pits in the soil surface for predation	

Habitat choice

All larvae made pits in the dry soil area. The minimum distance between the pit and the centre portion of the tray was 1.8 cm and the maximum distance was noted as 12.1cm (Fig. 22). From this study it is inferred that *M. pseudohyalinus* larvae prefer dry soil for pit building.

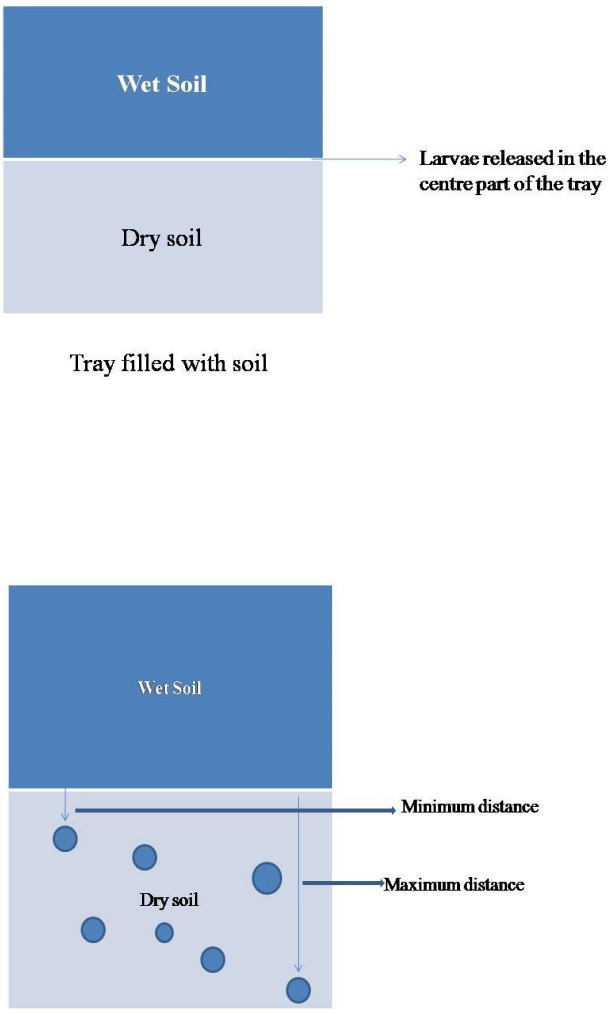


Fig. 22. Representation of pit building in wet and dry soil

2.5. DISCUSSION

This is the first study that documents the antlions and their ecology of southern India. *Myrmeleon pseudohyalinus*, a pit building species of genus Myrmeleon is a first report from India. Antlion larvae were collected from fifty study areas (Table 16) and according to the presence of pit building antlion; the sites were classified into four, namely abandoned areas (without any disturbances), human dwelling areas, forest boundaries and river banks. The most observed habitat was human dwelling area than other three habitats. It may be because of the presence of more protected areas from direct sunlight, rainfall and wind. Also there are lots of insects and small arthropods were plenty in human dwelling areas when compared to other areas. The human dwelling area comprises 54% of the total study area followed by abandoned areas (18%). The percentage of forest boundaries and river bank habitats in which the pit building antlion larvae observed was 14%. From this study it is also observed that the larvae prefer sand for making its pits and the percentage of sand in soil samples were 89.5%, 86%, 90% and 87% in abandoned areas, human dwelling areas, forest boundary areas and river banks respectively.

They are not only seen in these common habitats, but previous studies show the presence of larvae in sand dunes (*Myrmeleon hyalinus*) in Balkan Peninsula (Devetak *et al*., 2013). Here also soil and sand substrate were commonly found as pit building substrate, it may be because of the loose structure of the sand and dry soil which helps to make the steep conical pits and thereby enhances the predatory efficiency of this sit and wait predator. The study of Bozdogan and Satar (2017) show that the banks of wet lands are one of the most noted habitats of *Myrmeleon formicarius* larvae. This is true in the case of genus *Myrmeleon* which was collected from river banks also. Study by Pantaleoni *et al*., (2010) reported *M. mariaemathildae* were seen in dunes colonized by grassy vegetation which was a new habitat of pit building antlion larvae

From the field study, it was understood that the larvae made its pit in heap of sands, floor of abandoned buildings, shades of buildings (absence of direct sunlight), and shades of large trees like teak and coconut and also above ground level surface like terrace. The rainy season in the study area are prominent from June to September, a high number of adult antlions emerged before monsoon and the presence of adult antlions were more in early morning and evening. The

immature larval stages were present in both protected and unprotected habitats. Although antlions were considered as bioindicators of global warming and deserts, some small variations also seen in different species. Previous studies shows that *Cueta* species prefer warm, dry and lighted habitats where as *M. quinquemaculatus* prefers humid, cloudy areas (Bakoidi *et al.*, 2020), *M. caliginosus* seen in desert habitat, and *M. obscurus* was seen in proximity of buildings and sheltered conditions. *M. obscurus* and *quinqemaculatus* co- existing in the same habitat too (Badano, 2020). Comparison of pit building in protected and unprotected microhabitat of *M. brasiliensis* shows no difference in abundance, but density is increased in protected habitats and also positively correlated the pit size and larval size in both habitats (Lima, 2020).

Morphometric analysis helps to identify the size and shape variation of different species of a genus or same species in different geographical areas. The body measurements of larvae, cocoon and adult of genus *Myrmeleon* were carried out. The larvae of *M. pseudohyalinus* and *M. hyalinus* are the two species of antlion larvae morphometrically analysed and compared. The larvae of *M. pseudohyalinus* collected from seven study areas (Fig. 3) of four districts show similar body size (both second and third instar). The mean LBL, LBW, LHL, LHW and ML were 0.64 cm, 0.28 cm, 0.24 cm, 0.14 cm and 0.14 cm respectively in second instar larvae. In third instar larvae, the mean LBL, LBW, LHL, LHW and ML were 0.89 cm, 0.37 cm, 0.34 cm, 0.2 cm and 0.16 cm respectively.

Previous studies show that the size of same species in different places or different habitats may vary. The comparison of morphological variations of antlion larvae (*Myrmeleon hyalinus*) in Mediterranean and desert populations shows that the body size is larger in Mediterranean populations when compared with desert populations. It is also studied that the pit size is considered as a reliable feature for identifying particular instars in larval stage (Lewandowski *et al.*, 2004; Bozdogan *et al.*, 2013). In this study the larval instars were predicted by correlating the size of pit and inside dwelling larva, and found that the pit diameter and larval body width are positively correlated (Krishnan and Kakkassery, 2016).

The body measurements or size in various species of genus *Myrmeleon* was slightly different in various countries. Third instar larva of *M. caliginosus* has body length- 8.75mm, head length- 1.66 mm, head width- 1.27 mm, mandible length- 1.7mm, in desert habitat but in the case of *M.obscurus* the measurements as

follows, body length- 8.75mm, head length- 1.64 mm, head width- 1.2 mm, mandible length- 1.77mm and it is present in proximity of buildings and sheltered conditions. *M. quinqemaculatus* with a body measurements, body length- 15mm, head length- 3.32 mm, head width- 2.6 mm, mandible length- 3.43mm and has the same habitat seen in *M. obscurus* (Badano, 2020). Here, from this study it is understood that the third instar larvae of *M. pseudohyalinus* measures LBL- 9.9 mm; LBW- 4.6 mm; LHL- 3.2 mm; LHW- 1.9 mm; ML- 1.8 mm. For more accurate results more characters like distance between mandibles, mandible length, curved mandible length, mandible width, head length, head width, distance between mandible first and third tooth, distance between mandible second and third tooth, thorax length, thorax width, abdomen length and abdomen width (Scharf *et al.*, 2008) can be measured.

The body size has some relationship with its funnel shaped pit also. *Cueta sauteri* of Taiwan shows a positive correlation between larval body length and pit diameter in both field and laboratory conditions (Liang, 2010; Kross and Pilgrim, 2012). But from this study, the result shows a negative correlation between larval head length and pit depth and a postitive correlation between larval body width and diameter of its pits (Fig.6 and Fig. 7). Thus, an inference can be made from this correlation result that, the larval body width and length were the characters which decide the diameter of its pits**.** The pit diameter and depth are positively correlated (Krishnan and Kakkassery, 2016 and Liang, 2010), and helps the antlion by delaying the escape of prey which agree with the study of Kross and Pilgrim (2012). The study of Kitching (1984) shows a linear relationship between length of the *M. pictifrons* larvae and its pit diameter.

The knowledge about the size of cocoon (genus *Myrmeleon*) is a first study and regarding the size no references were available. However, this result gives a baseline data for the myrmeleontid fauna of Kerala. The mean circumference, diameter and width of the cocoon of genus *Myrmleon* are 2.6 cm, 0.8 cm and 0.1g respectively (Fig.12). The result of measurements of *M. pseudohyalinus* adult (Fig. 14) does not show much variation in the morphometry of different species of a genus. Here, the body measurements of *M. pseudohyalinus* were as follows, HTA- 24mm, FWL- 24mm, FWB- 6mm, HWL- 24mm, HWB- 5mm and AN- 4mm. However, the study of *Myrmeleon acerbus*, an Indian species (Kaur *et al.*, 2019), a member in the same genus showed slight difference and as follows, forewing

length 24.59- 24.76 mm, Forewing breadth 7.51- 7.62 mm, Hindwing length 21.86-21.92 mm, Hind wing breadth 6.02- 6.08mm.

The various abiotic parameters of the habitat of genus *Myrmeleon* were compared and correlated in this study (Table 19). Temperature, humidity, dew point, pressure, uv index, visibility and wind speed of study areas were checked and it was plotted against presence or absence of larvae, number of larvae and cocoon. The larvae show a significant negative correlation to dew point and a non significant negative correlation to humidity and uv index. Here, the mean dew point was 21ºC, mean humidity was 60.34% and mean uv index was 5.73. Dew point is the temperature at which water vapour content in air reaches the maximum point and the places with highest water content in air show a decrease in the larvae because the antlions prefer dry places. The uv index is moderate (5-6) in study areas (Mean UV index 5.73) irrespective of the different habitat. The mean temperature, humidity, dew point, pressure, UV index, visibility and wind speed observed were 30.60ºC, 60.34 %, 21.25ºC, 1010.45 millibars, 5.73, 11.56 and 10.07 km/h respectively.

Here the optimum temperature of *M. pseudohyalinus* lies between 30-32ºC, though antlions are shade loving and prefer dry conditions as well as low humid areas. The result of previous studies depicts that the *Myrmeleon immaculatus* larvae prefer high temperature than low temperature for frequent pit building (Arnett and Gotelli, 2001), not only the temperature influences the pit building of larvae, but also the optimum temperature (35ºC) helps to maintain the lowest level of mortality and shortest life cycle in *Myrmeleon obscures* (Bakoidi *et al.*, 2020). The study of Ngamo *et al.*, (2015) show that, the larvae prefer hot seasons than cool climate, the highest number of antlion were found at a temperature of 32ºC.

In the present climatic condition of Palakkad district, the highest number of pit and larvae (*M. pseudohyalinus*) were noted from January to March and April to June, the result agree with the study of Bozdogan and Satar, 2017. They studied the seasonal abundance of antlion larvae in Amanos Mountains, Turkey and they observed the maximum larvae in the month of May and June. From the field study it is understood that there is no relationship between pit size and soil temperature (Bozdogan and satar, 2017). Soil temperature has an important role in the pit making of *M. immaculatus* but, soil illumination has no significant effect on construction of pit. Studies by Klein (1982) inferred that the preference of shaded

areas by antlion larvae was not because of light, but in response to temperature. The pit size of *M. formicarius* increased with the increase in sand particle size, but altitude have no impact on pit diameter and sand particle size (Bozdogan *et al.*, 2013). *M.crudelis* larvae cease feeding below 20ºC (Lambert *et al.*, 2011). Also adults lost their mass very quickly when exposed to desert conditions (Scharf *et al.*, 2009b). Previous study also shows a negative correlation between rainfall and number of larvae in *Myrmeleon brasiliensis* (Freire and Lima, 2019). Scharf *et al.,* (2009b) shows a negative correlation between body size and habitat temperature and the higher temperature accelerates the duration of pupal stage. Annual rainfall and humidity were positively correlated with body size, these parameters increases the quantity of insect prey. Rainfall influenced the B*rachynemurus* larval behaviour, if heavy rain persists; they remain under the sand for 2-3 days (Cain, 1987).

The antlion larvae inhabited soil composed of sand, silt and clay particles. According to the International Union of Soil Sciences (IUSS), the soil particles were classified as follows and this was used to interpret the soil texture in this study. Sand particles has low water and nutrient holding capacity, loose when dry and very low stickiness when wet. The silt components has low to medium water and nutrient holding capacity and shows some stickiness when wet. The clay component has high water and nutrient holding capacity, hard when dry and high degree of stickiness when wet.

From the 27 soil samples collected from study areas, 21 samples were classified under texture class sand and 6 samples were coming under fine sand. The sand texture was analyzed by evaluating the sand, silt and clay content of the soil samples. The correlation between the components of soil and number of individuals were performed and the result shows a positive correlation between the numbers of larvae with sand having highest silt content. One reason behind the preference of sandy soil for pit building substrate was the nature of sand and silt with low water holding capacity and easily dry when wet compared to clay soil.

The study of Phogat *et al*., (2015) described that the larvae build its pits in sand particle with low water holding capacity and low stickiness when wet and the present study agrees with this result. For the survival of antlion larvae they choose the sand particle for making its pit. If the rainy season or wet condition occurs, the

larvae took a dormancy period by burying deep into the sand until the sand become dry. It is a survival mechanism of pit building antlion larvae.

Chemical composition of the media also has an important role in the pit building of antlion larvae. From this study, a negative correlation between calcium and potassium with the number of individuals were identified. The relationship between the chemical component of antlion inhabiting soil and its influence on larvae are not studied earlier and it can be studied in future. In general, higher the concentration of acidity, salinity, calcium and magnesium are suppressive to larval development. But potassium, sulphate and chlorides are good for larval development. Soils those are more favorable for the development of larvae are rich in potassium, magnesium and sulphates. The element that adversely affects the development of antlion is nitrogen (Maoge *et al.*, 2014).