A COMPARATIVE STUDY OF SPIDER DIVERSITY IN THREE DIFFERENT ECOSYSTEMS IN KUMBALANGHI, ERNAKULAM DISTRICT, KERALA

Dissertation submitted in partial fulfillment of the
Requirements for the degree of

Master of Science in Zoology

BY

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THE PHYLOGENETIC ANALYSIS OF GARDEN ANT

The project work submitted to Scared Heart College (Autonomous)
in partial fulfilment of requirement for the

Degree of Master of Science in Zoology

BY

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COCHIN- 682013

JUNE- 2019

SACRED HEART COLLEGE

DEPARTMENT OF ZOOLOGY

CERTIFICATE

This is to certify that project work entitled **"THE PHYLOGENETIC ANALYSIS OF GARDEN ANT"** is an authentic record of the research work carried out by Ms. **ARUNIMA VENU(Reg no: 17PZOO1784)** in the department as part of the M.Sc. Degree course during in year 2017-2019.

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Examiners:

Place: Thevara 1.

Date: 2.

CANDIDATE'S STATEMENT

I hereby declare that the work incorporated in the present dissertation is original and has not been submitted to any institution for the award of any diploma or degree.

I further declare that the results presented in the dissertation, consideration made therein, contribute to the advancement of knowledge of science.

Place: Thevara

Date:

Signature of Candidate

CERTIFICATE BY THE GUIDE

This is to certify that the contents of this dissertation entitled **"THE PHYLOGENETIC ANALYSIS OF GARDEN ANT"** is the original research work of Miss. ARUNIMA VENU carried out under my supervision.

I further certify that the work has not been submitted either partly or fully to any other University or institution for the award of any degree.

Dr. Smitha S.

Assistant professor

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CONTENTS

Appendix

Figure numbers	Figures
1	Isolated DNA
2	Amplification product
3	Blast result(a) Blast result(b)
4	Clustal result
5	Phylogenetic tree
6	ORF2
7	GenBank accession ID
8	*Crematogaster humile*
9	*Linepithema*
10	*Pachycondyla chinesis*
11	*Componotus*
12	*Paratechina longicornis*
13	*Brachymyrmex patagicornis*
14	*Myrmica rubra*
15	*Formica*
16	*Polyrhachis paracamponotus*

ABSTRACT

The order Hymenoptera includes, ants, wasp, and bees, among them ant are omnipotent in the ecosystem, except in ice cover regions. Due to their eusocial behaviour, polymorphism in females, restricted reproduction, brood care etc., led them to survive in the land. They do various functions and are of great significance to the environment. They are the most diverse species on earth. Several studies were already reported in the colony formation and behavioural activities. The Polyrhachis and Componotus are widely found in the garden, which created a curiosity and hence, tried to find the phylogeny of the ant. In the present study specimen was collected. DNA isolated from legs and amplified using primers lepF1 and lepR1 (partial mitochondrial gene). Then the sequencing of the amplicons was done using ABI3730XL cycle sequencer, followed by bioinformatics analysis. The phylogenetic study helps to trace the evolutionary relationship of the ants based on the database available in NCBI.

INTRODUCTION

Ants are part of the class insecta belong to the phylum Arthropoda. Ants are described as eusocial insect, with head, thorax, and abdomen, waterproof body, which is made of chitin. They can carry ten times their weight. They have compound eyes, which is made of omatidia, which detects light and shadow. The two antennae present are used to detect their nest mates and enemies. When they find food, they emit pheromones that provide scent trails so their nest mates can locate to it and maxillary palp present also detects the scent. The mandibles are used for cutting and biting, and also to carry and grasp. The legs are attached to the thorax, has six legs and their vital organs and reproductive organs are present in the abdomen, thus is called as gaster. The formicinae subfamilies have an acidopore to emit formic acid when threatened. They breathe through spiracles, present in body and take in oxygen and give out carbon dioxide. Their heart is tube like, in which colourless blood flows from the head to other body parts and then flows back to head. The nervous system consists of long nerve chord which runs from head to rear. The ants possess a segment called petiole, which connect thorax to abdomen. This feature is used to identify the subfamily (https://harvardforest.fas.harvard.edu/ants/about-ants).

The ants are divided into three castes: reproductive females, reproductive males, and non-reproductive female [https://www.antkeepers.com/facts/ants/caste-system-ant-societies/]. The females are polymorphic, represented by queen, worker, and soldiers. These caste is very efficient and is necessary for the well-functioning of the society, as these caste perform different functions, like a few for caring young ones, building the nest, foraging food and queen to produce young ones. In some groups, queen are dominant and lays egg, while in some the reproductive dominance is overtaken by fertile workers called gammergates. The ants feed mostly on almost all things, and are referred to as omnivorous. Restricted reproduction in females helps to sustain their population. The different types of ants present, exhibit difference in behaviour, nest building patterns, but all of them shows social nature , such they work to

maintain their society. Many studies have been conducted to learn their social behaviour. The male ants are haploid, while female are diploid, is produced by mating.

Since, they play a very important role in ecosystem, as predators, symbioses, controls pest, pollinators of flowers and plant, and turns the soils, providing sufficient aeration, as earthworm does.

The DNA barcoding of the mitochondrial genome helps, one to trace the evolutionary history of an animal. The mitochondrial genome contains the genetic marker region such, as cytochrome c oxidase, which is used, widely. This technique helps to identify, species easily than the conventional taxonomic method, in which one requires profound knowledge and expertise in this arena. Now days, this method is applied in a varied field, like identifying species diversity, relation to the environment, presence of certain bacteria. For, the purpose DNA of the specimen is prepared and amplified using the PCR. The template is purified and sequenced. Obtained sequence is, compared with the other sequence present to find their evolutionary relationship and a phylogenetic tree is constructed.

Species identification based on sequencing of standard genetic markers — DNA barcoding — is now well established. The so-called ‘Folmer region’ of the cytochrome oxidase I (COI) gene of the mitochondrion has been widely accepted as the standard barcoding [1]. The Indian subcontinent is highly bio diversity rich, with varied environments and habitats. It was found, that 828 species and subspecies of ant was found, belonging to 100genera placed in 10 subfamilies. Among them, Formicidae is the second species rich group [2].

REVIEW OF THE LITERATURE

Ants (Hymenoptera: Formicidae) are the most successful group of insects in the world, are more diverse consisting of more than 2000 species [3]. They exhibit varied behaviours, colony structures and genetic system, the evolution have always surprised the evolutionary biologist. They directly or indirectly affect other organisms, as they possess strong ecological foot print in most terrestrial ecosystem [4]. They are the first group of predatory insect to become eusocial [5]. They play a key role in shaping the ecosystem and function, as they are dominant taxa in terrestrial ecosystems [6]. Several lineages comes under, the order Hymenoptera, which includes ants, bees, and aculeate wasps, that have been independently evolved to become eusocial animals. The characteristic feature of this eusocial lineage is the division of labour, cooperative brood care, an overlapping generations [7].

They interacts with organisms from different trophic level and many ants species exhibit cryptic behaviour, like live in underground, leaf litter, or in canopies of the trees, which, makes their study difficult. Many species can live together in a given stratum. The ants are commonly omnivorous feeding on different food items, incorporating live prey, dead arthropods, seeds and plant exudates. Very few exhibit, a slight variation in food habits such as, fungus cultivators and predates exclusively on arthropods. The fatty acids from diet are directly or indirectly present in the ants, which can change or remain without any change, which depends on environmental disturbances. The fatty acid present in the ant enable us to know their relationship [8]. The ecological and evolutionary success of the more than 15,000 described extant ant species (http://www. antweb.org) is often attributed to their sociality and ability to engineer environments, e.g., by building elaborate nests, tending aphids for honeydew, or practicing sustainable agriculture [9]. This animal exhibit high division of labour and only a small portion of them reproduce and maintain to their society. The work ant do depends on their caste [10]. The number of queens per colony varies a great deal between species and sometimes even within species [11]. The ants uniquely possess Social insect genome preserves regulatory region, which is imparts sociality. While, female ants display a wide variety of morphological castes, including workers, soldiers, ergatoid (worker-like) queens and queens. Alternative caste development within a species arises from a variable array of genetic and

environmental factors, genetic and environmental factors plays an important .Castes themselves are also variable across species and have been repeatedly gained and lost throughout the evolutionary history of ants. The female morphology varies as a function of size, such that larger individuals possess more queen-like traits. Thus, the diverse mechanisms that influence caste development are simply mechanisms that affect size in ants. Each caste-associated trait has a unique relationship with size, producing a phenotypic space that permits some combinations of worker- and queen-like traits, but not others. These castes are gained and lost by modifying the regions of this phenotypics pace that are realized within a species. Species gain or lose castes over evolutionary time either by modifying this size– frequency distribution or by modifying the relationship between tissue growth and size. DNA methylation has been implicated in regulating gene function in social insects [12]. There are several morphological evidences for sociality in ants, they uniquely possesses specialized, complex, metaplueral gland, external opening on the posterior region of the mesosoma, the secretion from the glands are found to aid in defence in social interactions and colony hygiene. The modern works on phylogeny of ants dates back to division of the Formicidae into two subfamilies- Poemoid complex consisting of Ponerinae, Cerapachyinae, Myrmicinae, Dorylinaeand, Letanillinae, and Themyrmecoid complex having Myrmecinae, Pseudomymerciinae, Dolichoderiinae and Formiciinae[13]. Polyrhachis subgenera, including the presence of both pronotal and mesonotal spines, a columnar petiole with a pair of hook-shaped spines [14]. As of 2013, 21 subfamilies have been recognised, among them four are dominant in terms of geographic spread, population density, and species diversity: Ponerine, Dolichoderinae, Myrmicinae, and Formicinae, among the Formicinae consists of 3,794 known species [5]. They shows robust communication system, by chemical communication [16]. Above the ground, from several meters high to the canopy, the formicinae and Dolichoderinae dominate. Natural selection drives the evolution of ant life cycles [17].

Ants occupy a special position in ecosystem, their social habit, high species richness and large biomass makes them a good model of study behaviour ecology and because of their terrestrial dominance, they become important components of the environment [18].

Ants are among the leading predators of invertebrates in most ecosystems and are also prominent herbivores in many Neotropical communities. Various ant species participate in symbiotic relationships with some plants and animal's ecological dominance requires a robust phylogeny of their early evolution and a reliable timescale for their diversification. However, both the age of ants and the relationships among their earliest evolving lineages remain controversial [19, 20].They act as ecological indicators and ecosystem engineers. One potential solution to this problem may be using DNA barcoding as an inventory tool. If the ant species from an area have been DNA barcoded, even without assigning scientific names, the barcodes of future ant collections can be compared to the DNA barcode library for the island and adjacent mainland area (which, unfortunately, may be as large as the entire world). Such faunistic comparisons can be made with any kind of tissue, rather than requiring tissue from a particular morph or life stage [21]. The barcodes of the future collection can be compared with DNA barcode library [22]. A total of 828 valid species and subspecies names belonging to 100 genera are listed from India [23]. Particularly, in studies focused on the dynamics of kin selection, with in colony conflicts of interest, caste differentiation, and division of labour.

Today ants occupy keystone positions in most terrestrial environments, serving as major conduits of energy and organic material. They are, for example, important turners of the soil, matching or exceeding the activity of earth worms in this role. They are among the leading predators of invertebrates in most ecosystems, and in the Neotropics they are the leading herbivores as well, with leaf-cutter ants taking more than15% of the fresh vegetation. Interactions with ants have shaped the evolution of diverse organisms to an astonishing degree. Ants participate in symbioses—some facultative, some obligate—with over 465 plant species in over 52 families, with thousands of arthropod species, and with as-yet-unknown numbers of fungi and microorganisms. Clearly, the study of most ecosystems must include the study of the resident ant species. Because of their complex colony-level behaviours, ants serve as, model organisms for the highly visible disciplines of behavioural ecology and socio-biology, particularly in studies focused on the dynamics of kin selection, with in colony conflicts of interest, caste differentiation, and division of labour. Moreover, ant plays an important role in tropical and subtropical region, as consumers and ecosystem engineers. They reduce the predators and they interfere with their

behaviour and ant foraging can influence the flower, visitation behaviour of pollinators through direct and indirect behaviour [24].

The study of ant in environment is so, relevant because, the presence of invasive species in island and provides insight to biogeography of the particular place [25].

They have strong ecological footprint in terrestrial communities, are found to directly or indirectly influence many other organism. They tend to show, varied array of behaviour, genetic, and evolutionary systems [26].

The degree of queen and worker is found to be increased in some lineages[27], but while in some intermediates and super individuals are present which is stands intermediate to the morphological appearance of queen, worker, and male. The queen present has ability to control the development of the caste, as the reproductive queen is known to inhibit the reproduction of new queen in number of ant species. The maternal factors present in the eggs, are found to influence the adult [28].

India is one of the mega-diversity regions, for ants. Where, they occur frequently throughout the country. Ants are important component of ecosystem [29]. The social insects, genome exhibit dramatic evolution in gene composition and regulation while preserving regulatory features [30]. The study of ant genome provides insight, into social insect biology [31].

The social behaviour ants are shows are contributed by particular genotype, in locus of allozymes [32]. The current taxonomic methodology is insufficient to describe the growing levels of diversity in both a standardised and general way due to the limitations of using only morphological traits to describe. Thus, the classification of can be done in an easiest way [33].

Since 2003 DNA based identification of animal life was possible, by encoding the 5' end of mitochondrial region of cytochrome C oxidase 1[34]. Before, half a century the works on the ant phylogeny had been improving, including the information taken from the morphology of the living fauna. The information supplemented with morphological characters by fossil evidences, along with the DNA sequence data had gave a boost to ant phylogeny [35].

Preliminary comparisons revealed that these COI primers generate informative sequences for phylogenetic analyses at the species and higher taxonomic levels [36]. The use of morphology along with molecular studies help us to know about the cryptic species and it act as a tool for identification of different species, beyond the morphological differences [37].

The mitochondrial DNA is not fool proof method for barcoding and identification. As this relies on low level of mDNA sequence, to find variation within species as, compared with between the species. The maternally inherited DNA marker would be unreliable if male and female have different lines of histories within the species. But, there is a chance of nuclear genome been integrated into mDNA, with the use of conserved primers. Relying, more on the CO1 cannot be used as marker for taxonomic identification, because of the presence of pseudo genes in mDNA and its inconsistent evolutionary rate lead to disadvantage [38].

Also there are many hypotheses to find the phylogenetic positions of bees and ants placing in the phylogenetic analysis [39].

Discrimination of closely related species using DNA barcodes often relies on differences in one or a very few base-pair sites. The sequences reported in BARCODE records are interpretations of the raw data produced by sequencing systems, so the post-processing of these data are critical elements in determining the accuracy and reliability of the sequences themselves. The quality scores of, and degree of the overlap between, the bidirectional sequencing runs are therefore important metadata in the interpretation of analyses of barcode data [40].The identification of species is often difficult. The use of DNA barcodes, short DNA sequences from a standardized region of the genome, has recently been proposed as a tool to facilitate species identification and discovery [41].

They act as ecological indicators and ecosystem engineers. One potential solution to this problem may be using DNA barcoding as an inventory tool. If the ant species from an area have been DNA barcoded, even without assigning scientific names, the barcodes of future ant collections can be compared to the DNA barcode library for the island and adjacent mainland area (which, unfortunately, may be as large as the entire world). Such faunistic comparisons can be made with any kind of tissue, rather

than requiring tissue from a particular morph or life stage [42, 43]. The barcodes of the future collection can be compared with DNA barcode library.

MATERIAL AND METHODS

Sample collection

The sample used for study was the black ants, caught from the garden. They had been hand-picked from the soil, in Chottanikkara.

DNA isolation

1. The leg of the ant was taken and homogenised with 500µL lysis buffer (100mM NaCl, 100mm Tris-Cl 25mM EDTA, 0.5% SDS)

2. Incubated at 60 º for 30 minutes.

3. Added equal volume of chloroform/ isoamyl alcohol mixture (24:1 ration), (960µL chloroform and 40µLisoamyl alcohol)

4. Mixed by inverting the tubes

5. Centrifuged at 12000 rpm for 10 minutes.

6. Collected the aqueous phase, transferred to new tube and added 0.7 volume of isopropyl alcohol. (If aqueous phase is 1mL add 0.7 mL isopropyl alcohol. If aqueous phase is 500µL, add 350µLof isopropyl alcohol)

7. Mixed by inverting the tubes

8. Centrifuged at 12000 rpm for 10 minutes.

9. Retained the pellet after, carefully decanting the isopropyl alcohol

10. Added 500µL of 70% ethanol

11. Retained the pellet within opened tubes till the ethanol smell vanishes.

12. Added 30µL of nuclease free water to the sample.

AGAROSE GEL ELECTROPHORESIS

1. Preparation of 1X TAE buffer

To prepare 500mL 1X TAE buffer, added 50mL of 10 X TAE buffer to 450mL of sterile distilled water and mixed well.

Composition: Tris, glacial acetic acid and EDTA

To prepare 1L of 10X solution

A. Weighed 48.5g Tris
B. Measured 11.4mL glacial acetic acid
C. Taken 20 mL 0.5 EDTA (pH 0.8)
    a) Dissolved Tris buffer in about 800mL of deionized water.
    b) Added acetic acid and EDTA.
    c) Added deionized water to 1L.
    d) Dilute the stock solution 10:1 to make a 1X working solution.

2. Preparation of 1 % agarose gel.
3. Add 60mL of 1X TAE buffer to 0.6 g agarose gel.
4. Melted the agarose in microwave oven, until agarose got dissolved completely, by occasionally swirling the flask.
5. Allowed the solution to cool, to about 55- 60 º C.
6. Added 2µL EtBr and pour this to gel tray.
7. Allow the gel to solidify for 30 minutes at room temperature.

LOADING THE DNA SAMPLE

To prepare sample for electrophoresis, add 2µL of Bromophenol blue (dye) and

10 µL sample. By pipette, mixed well the contents and load the sample onto the gel.

ELECTROPHORESIS

Connected the power to electrophoretic power supply red- anode & black – cathode) Electrophoresised at volts and 90 mA until dye markers have migrated to an appropriate distance, depending on the size of the DNA and visualised under UV trans- illuminator.

PCR

Polymerase chain reaction is an in-vitro method of enzymatic synthesis of specific DNA sequences. The technique was developed by Kary Mullis in 1983.

Procedure

a. Template DNA-1µL
b. 10X as a buffer – 2.5 µL
c. dNTPs -1µL.
d. taq DNA polymerase – 0.5 µL .
e. Forward primer - 1µL
f. Reverse primer -µL

Make up to 25 µL with DNase free water. Total reaction volume is 25 µL.
Carried out the amplification in the thermo cycler, Mini Amp plus Thermal cycler (Applied Bio system).

The primers used for PCR is LepF1(5'-ATTCAACCAATCATAAAGATATTGG-3') and LepR1(5'-TAAACTTCTGGATGTCCAAAAAATCA-3').[44]

PCR Amplification

Step 1- Initial denaturation it is generally performed at 98ºC for 1-3 minutes, depending on the GC content of the template. Initial denaturation performed only once at the beginning of the reaction.

Step 2- Denaturation steps are performed for 10 second at 98º C for 35 cycles. During this step two strands of DNA melts open to form a single stranded DNA and all the enzymatic reaction stops.

Step 3- Annealing

The annealing temperature is 49º C for 30 seconds, during this step annealing of primers occurs.

Step 4 – Extension

Primer extension, resulting in synthesis of a new DNA strand is carried out at 72º C for 45 seconds. Extension time is determined by the length of the sequence to be amplified.

Step 5- Final extension

In final extension, the enzyme is allowed to finish any incomplete synthesis by Carrying out final extension at 72ºC for 5-

PCR used for amplification was

1. Following PCR amplification, mixed 2 µL of template and 1µL gel loading buffer.

2. Mixed thoroughly.

3. Carefully pipetted mixture and loaded in the well of the 2%agarose gel.

4. Load 2 µL of the ready to use 100bp ladder provided. Note down the order in which the samples have been loaded.

5. Electrophoresed the sample for 45 minutes.

6. Visualized the gel under UV-trans illuminator

Protocol steps:

GEL PURIFICATION

1) Diluted the sample with DNA clean-up binding buffer. Mixed well by pipetting up and down or flicking the tube. Do not vortex. For diluted samples larger than 800µL, load a portion of the sample, proceed with step2, and repeat as necessary.
2) Inserted column into collection tube and loaded samples onto column. Spined for 1 minute, then discarded flow- through.
3) Re- inserted column into collection tube. Added 200µL DNA wash buffer and spin for 1 minute. Discarded flow – through is optional.
4) Repeated step 3.
5) Transferred column to a clean 1.5 mL microfuge tube.
6) Added 6µL of DNA elution buffer to the centre of the matrix. Waited for 1 minute and then spined for 1 minute to elute DNA. Typical elution volumes are 2-20 µL. Nuclease – free water (pH7- 8.5) can also be used elute the DNA.

SEQUENCING

Sanger sequencing was performed with forward and reverse primers in ABI3730XL cycle sequencer.

SEQUENCE ANALYSIS

Sequencher software 5.4.6 (Gene Codes Corporation, Ann Arbor, Michigan), is a premier software choice for DNA and RNA sequence assembly and analysis. Sequence similarity is done to trace the biological significance of nucleotide sequence. For that require two or more sequence. A database (nucleotide) search gives us a sequence that matches our query sequence. Sequence comparison is the most powerful and reliable method for determining evolutionary relationship between gene.

CONTIG GENERATION

Steps

1. Imported both forward and reverse sequence to the project window.

2. Low quality bases are trimmed.

3. Assembled both the sequences, to generate contig.

BLAST

Basic Local Search Alignment Tool (BLAST) is a tool for similarity search. BLAST performs local alignment.

Here, in our work we conducted BLASTN, which compares a nucleotide query sequence against a nucleotide sequence database.

Procedure

1) Assessed the BLAST home page (http;//blast.ncbi.nlm.gov/blast.cgi) and choose ‘nucleotide blast’ (blastn) clicking on the link, since this is the blast program we need, to search for nucleotide sequences that match our query sequence.

2) Pasted our nucleotide sequence in fasta format in the query box. We may instead use the GenBank ID or Accession number of the sequence, as the query, if the information. Change the default parameters if necessary and click ‘BLAST’.

3) The BLAST result page showed the hits and the colour codes indicating their different percentage of similarity with the query.

4) We may click on the link and obtain the sequence that showed maximum similarity with our sequence and look at the areas of similarity between the two.

MULTIPLE SEQUENCE ALIGNMENT

Multiple sequences can be compared and aligned using multiple sequence alignment, provides information on the conserved regions of the DNA.

Clustal W

ClustalW is a widely used for aligning a number of homologous nucleotide or protein sequence. The sequence is progressive. Trees can be constructed from alignments.

1. Compute the pairwise alignment for all the sequence.

2. Store the similarities in a matrix.

3. Converts sequence similarity matrix value is converted to distance measure, reflecting evolutionary distance between each pair of sequence.

4. Constructs a tree (guide tree), for the order in which pairs of sequence are to be aligned and combined with previous alignment with. Using the tree obtained, the first node is aligned to the second node. After fixing the alignment, another sequence is

aligned. When there is alignment in between the two groups or when there is alignment in between that had the highest alignment score.

PHYLOGENETIC ANALYSIS

Phylogenetic analysis is the study of an organism with respect to its related neighbours.

Steps

1 .Obtaining DNA sequences.

2. Building of multiple sequence alignment.

3. Converting the alignment data to a phylogenetic tree.

RESULTS

DNA

DNA was isolated: concentration of the DNA measured by Qubit-50µg/µL.

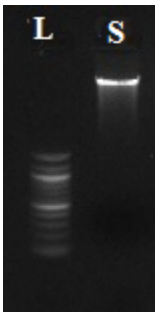

Fig (1): The DNA was isolated.

Amplification

The DNA was used for amplification and obtained the amplicon at 48ºC.

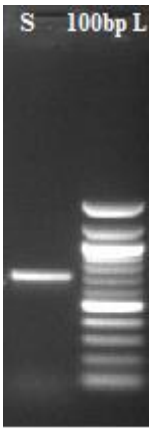

Fig (2): Amplification product

Contig

After purification the amplicons were sequenced with specific primers and quality sequence of Q20, 99%percent was obtained.

Sample (S):

>CTCAATAAGAATAATTATTCGATTAGAACTAGGTTCGCCTAACTCATTA
ATTCTTAATGACCAAACTTTTAATTCCATTGTTACAAGACATGCTTTTATT
ATAATTTTTTTTATAGTTATGCCATTCATAATTGGGGGATTTGGAAATTTT
TTAGTTCCTCTAATAATTGGATCTCCTGATATAGCATACCCTCGTATAAAT
AACATAAGATTCTGACTTCTACCCCCTTCAATCTCCTTACTACTTATAAGT
AATTTCATTAATGAAGGATCGGGGACAGGATGAACTGTTTATCCCCCCCT
GGCATCAAACTCATTCCACAGGGGCCCATCAATTGATCTAACCATTTTTT
CCCTTCATATTGCTGGAATATCATCAATCCTAGGGGCTATCAATTTTATCT
CCACTATTATAAATATACATAACTCTAACATCACTATAGATAAAATCCCC
CTATTAGTATGATCTATTCTCATTACAGCTATTCTTCTTTTACTGTCTCTCC
CAGTATTAGCCGGAGCTATTACCATATT

The blast annotation shows 88.66% identity with *Ploryrhachis paracomponota* with 0.0 E value and 99% query coverage according to the nucleotide homology.

Lineage:

Lineage (full): cellular
organisms; Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Protostomia; E cdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insect a; Dicondylia; Pterygota; Neoptera; Holometabola; Hymenoptera; Apocrita; Aculeata ; Formicoidea; Formicidae; Formicinae; Camponotini; Polyrhachis

Blast results

RID DTUUS3C8015 (Expires on 05-17 12:47 pm)

Query ID	lcl|Query_84747	**Database Name**	nr
Description	None	**Description**	Nucleotide collection (nt)
Molecule type	dna	**Program**	BLASTN 2.9.0+
Query Length	534		

Graphic Summary

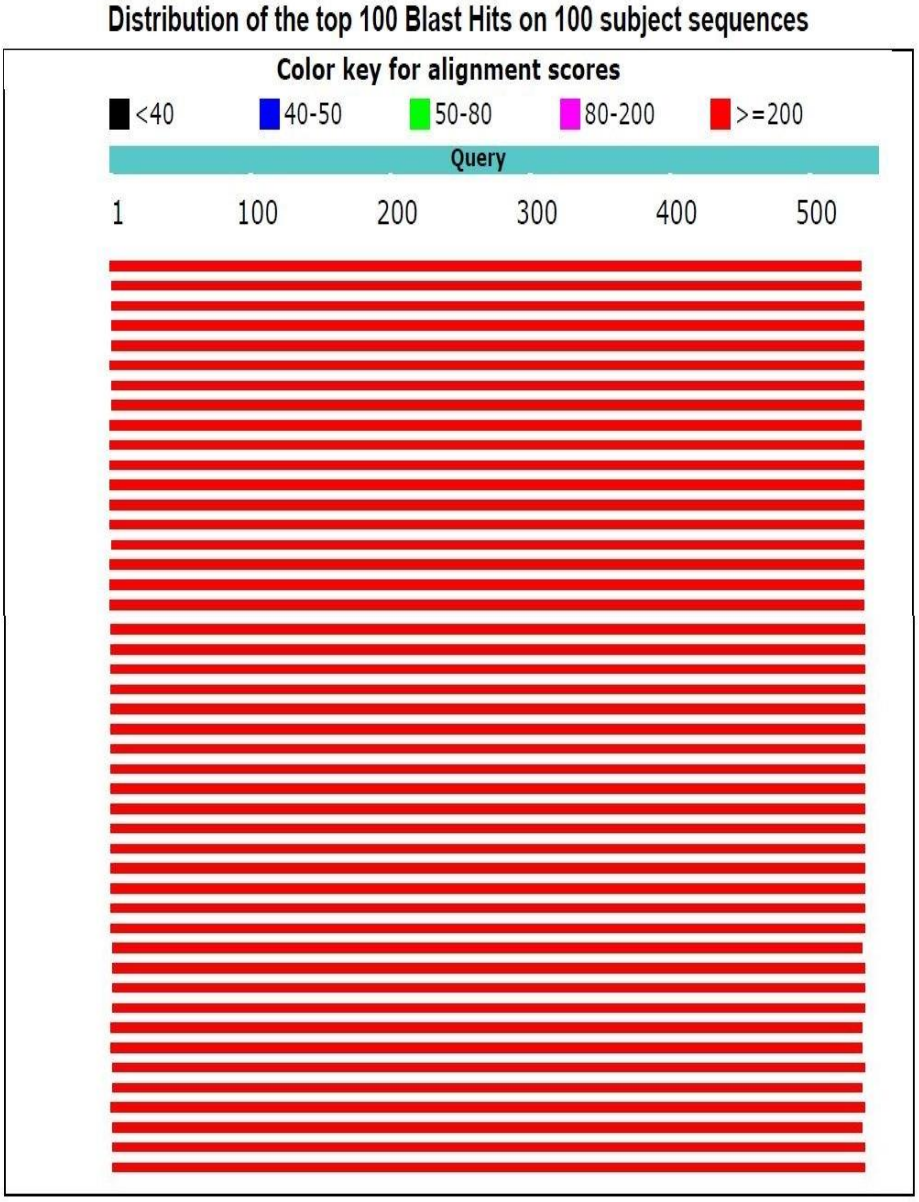

Fig.3: Blast result (a)

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Polyrhachis paracamponota isolate GXJX0009 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	678	678	99%	0.0	89.66%	JQ681070.1
Camponotus parius voucher AAI_06 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	649	649	99%	0.0	88.70%	KC685012.1
Camponotus sp. MG054 voucher CASENT0062411-D01 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	547	547	99%	1e-151	85.18%	HM373080.1
Camponotus sp. MG064 voucher CASENT0053845-D01 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	547	547	99%	1e-151	85.18%	DQ176210.1
Camponotus sp. MG047 voucher CASENT0060494-D01 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	531	531	99%	1e-146	84.62%	KF200290.1
Camponotus christi ferrugineus voucher CASENT0050413-D01 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	518	518	100%	1e-142	84.14%	HM373063.1
Camponotus sp. MG047 voucher CASENT0148213-D01 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	514	514	99%	1e-141	84.05%	KF200433.1
Camponotus sp. MG047 voucher CASENT0135816-D01 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	514	514	99%	1e-141	84.05%	KF200278.1
Polyrhachis sp. FMNH-INS 2842194 voucher FMNH-INS_2842194 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	512	512	99%	5e-141	84.05%	KM348307.1
Camponotus aff. christi 01 MAS-2013 voucher CASENT0144692-D01 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	510	510	100%	2e-140	83.93%	KF200827.1

Fig.3: Blast result(b)

CLUSTAL RESULT

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clustalw.aln

CLUSTAL 2.1 multiple sequence alignment

Polyrhachis_paracamponota      CTCAATAAGAATAATTATTCGTTTAGAATTAGGTTCGCCCAACTCACTAA
Camponotus_parius              -TCAATAAGAATAATTATTCGATTAGAACTCGGTTCACCCAACTCATTAA
Sample                         CTCAATAAGAATAATTATTCGATTAGAACTAGGTTCGCCTAACTCATTAA
Camponotus_sp.                 -TCAATAAGAATAATTATTCGATTAGAACTTGGATCTCCTAATTCCTTAA
Polyrhachis_sp.                CTCTATAAGAATAATTATCCGTTTAGAACTTGGCTCTTCTAATTCATTAA
Camponotus_christi             CTCAATAAGAATAATTATTCGATTAGAACTTGGATCTCCTAATTCATTAA
                                ** ************** ** ****** * ** **  * ** **  ***

Polyrhachis_paracamponota      TTCTCAATGATCAAACTTTTAACTCTATTGTCACAAGACATGCTTTTATC
Camponotus_parius              TTCTTAATGATCAAACTTTTATCCCATTGTTACAAGACATGCTTTTTTC
Sample                         TTCTTAATGACCAAACTTTTAATTCCATTGTTACAAGACATGCTTTTATT
Camponotus_sp.                 TTTTAAATGACCAAACATTTAATACTATTGTCACAAGACATGCTTTTATT
Polyrhachis_sp.                TTAATAATGATCAAACTTTTAACTCCTTAGTTACAAGACATGCCTTTATT
Camponotus_christi             TTCATAATGATCAAATCTACAATACTATAGTTACAAGACATGCTTTTATT
                               **   ***** **** *   *  *  * ** ********** *** *

Polyrhachis_paracamponota      ATAATTTTTTTTATAGTTATACCATTTATAATTGGAGGATTTGGAAATTT
Camponotus_parius              ATAATTTTTTTTATAGTTATACCATTCATAATTGGAGGATTTTGGAATTT
Sample                         ATAATTTTTTTTATAGTTATGCCATTCATAATTGGGGGATTTGGAAATTT
Camponotus_sp.                 ATAATTTTTTTTATAGTTATGCCATTCATAATTGGGGGTTTTGGAAATTT
Polyrhachis_sp.                ATAATTTTTTTTATAGTAATACCATTTATAATTGGGGGATTTGGAAACTT
Camponotus_christi             ATAATCTTCTTCATAGTTATGCCCTTTATAATTGGAGGATTTGGAAATTT
                               ***** ** ** ***** ** ** ** ******** ** *** * ** **

Polyrhachis_paracamponota      CTTAGTGCCTCTGATAATTGGATCTCCTGATATAGCATACCCTCGTATAA
Camponotus_parius              CTTAGTACCTTTAATAATTGGATCCCCTGACATAGCATACCCTCGTATAA
Sample                         TTTAGTTCCTCTAATAATTGGATCTCCTGATATAGCATACCCTCGTATAA
Camponotus_sp.                 TTTAGTTCCACTAATATTAGGGTCTCCTGATATAGCATATCCTCGTTTAA
Polyrhachis_sp.                TTTAGTTCCATTAATGTTAGGATCACCTGATATAGCATACCCTCGTATAA
Camponotus_christi             TCTAGTCCCTTTAATATTAGGATCTCCAGATATAGCTTTCCCTCGAATAA
                                **** **  * **  * ** ** ** ** ***** *  *****  ***

Polyrhachis_paracamponota      ATAACATAAGATTCTGACTCCTACCCCCATCAATTTCTTTATTACTCCTA
Camponotus_parius              ATAATATAAGATTTTGACTTTTACCTCCATCAATTTCTTTACTACTCTTA
Sample                         ATAACATAAGATTCTGACTTCTACCCCCTTCAATCTCCTTACTACTTATA
Camponotus_sp.                 ATAATATAAGATTTTGACTTCTACCACCTTCAATCTCTTTATTAATCTTA
Polyrhachis_sp.                ATAATATAAGATTTTGACTTCTTCCCCCCATCAATTACTTTACTAATTTTA
Camponotus_christi             ATAATATAAGATTCTGACTTCTTCCCCCTTCAATTTTTTTATTAATTTTA
                               **** ******** *****  * ** ** *****     *** ** *  **

Polyrhachis_paracamponota      AGTAACTTTATTAATGAAGGATCAGGAACAGGATGAACTGTTTATCCGCC
Camponotus_parius              AGTAATTTTATTAATGAAGGAGCAGGAACAGGATGGACTGTCTATCCCCC
Sample                         AGTAATTTCATTAATGAAGGATCGGGGACAGGATGAACTGTTTATCCCCC
Camponotus_sp.                 AGTAATTTTATTAATGAAGGATCAGGAACAGGTTGAACAATTTATCCCCC
Polyrhachis_sp.                AGTAATTTTATTAATGAAGGATCGGGTACAGGTTGAACAGTTTACCCTCC
Camponotus_christi             AGAAATTTTATTAACGAAGGATCTGGGACAGGTTGAACTGTCTACCCCCC
                               ** ** ** ***** ****** * ** ***** ** **  * ** ** **

Polyrhachis_paracamponota      CTTGGCGTCCAACTCATTTCACAGAGGCCCATCAATCGACCTAACTATCT
Camponotus_parius              TTTAGCATCTAACTCATTTCATAGAGGCCCATCAATTGATCTAACTATTT
Sample                         CCTGGCATCAAACTCATTCCACAGGGGCCCATCAATTGATCTAACCATTT
Camponotus_sp.                 TTTAGCATCTAATTCCTTTCATAGTGGACCATCTATCGATCTTACTATCT
Polyrhachis_sp.                TTTAGCATCAAATATTTTTCATAGAGGCCCTTCAATTGATCTCACTATCT
Camponotus_christi             TCTATCATCTAATACATTTCACAGAGGGCCCTCTGTTGACCTAACTATTT
                                *  * ** **    ** ** ** ** ** **  * ** ** ** ** *

Polyrhachis_paracamponota      CCCTCTTCTAGTATGATCTATCCTTATTACAGCTATTCTTCTTCTACTAT
Camponotus_parius              TCCCTTATTAGTATGATCAATTTTATTTACAGCAATTCTTCTTTTACTAT
Sample                         CCCCCTATTAGTATGATCTATTCTCATTACAGCTATTCTTCTTTTACTGT
Camponotus_sp.                 ACCTCTATTAGTTTGATCTATTCTTATTACAGCTATTCTTCTTCTTTTAT
Polyrhachis_sp.                TCCTTTATTAGTCTGATCTATTCTTATTACTGCTATTCTTCTTCTTCTTT
Camponotus_christi             TCCTTTATTAGTATGATCTATTCTTATTACAGCAATTCTTCTTCTTCTTT
                                **  *  **** ***** **  *  **** ** ********* *  * *

Polyrhachis_paracamponota      CTCTCCCAGTCCTAGCTGGAGCTATTACCATA--
Camponotus_parius              CTCTCCCAGTTCTAGCTGGAGCTATTACCATA--
Sample                         CTCTCCCAGTATTAGCCGGAGCTATTACCATATT
Camponotus_sp.                 CCCTTCCTGTTTTAGCAGGAGCAATTACTATATT
Polyrhachis_sp.                CTCTCCCAGTTTTAGCAGGAGCAATTACTATA--
Camponotus_christi             CTCTCCC-GTTCTAGCAGGAGCTATTACTATATT
                               * ** ** **  **** ***** ***** ***
```

```
clustalw.dnd

(
(
Sample:0.04848,
(
Camponotus_sp.:0.08432,
(
Polyrhachis_sp.:0.08396,
Camponotus_christi:0.08333)
:0.02077)
:0.02702)
:0.00132,
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Camponotus_parius:0.07287);
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Fig.4: Clustal result

Phylogenetic tree

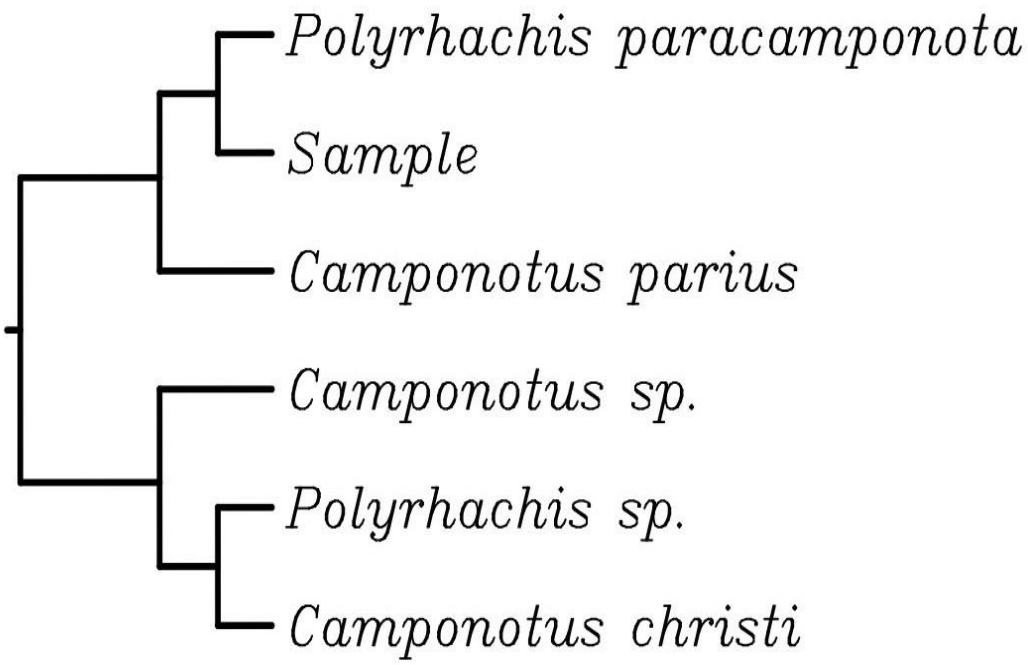

Fig.5: Phylogenetic tree

GenBank submission

ORF (open reading frame) Finder was used to find the coding region in the sample sequence. Here, ORF 2 contains 414 nucleotide which codes for 137 amino acid and this region was used for GenBank submission. The sequences from (Start): 119 to Stop : >532 is the open reading frame taken.

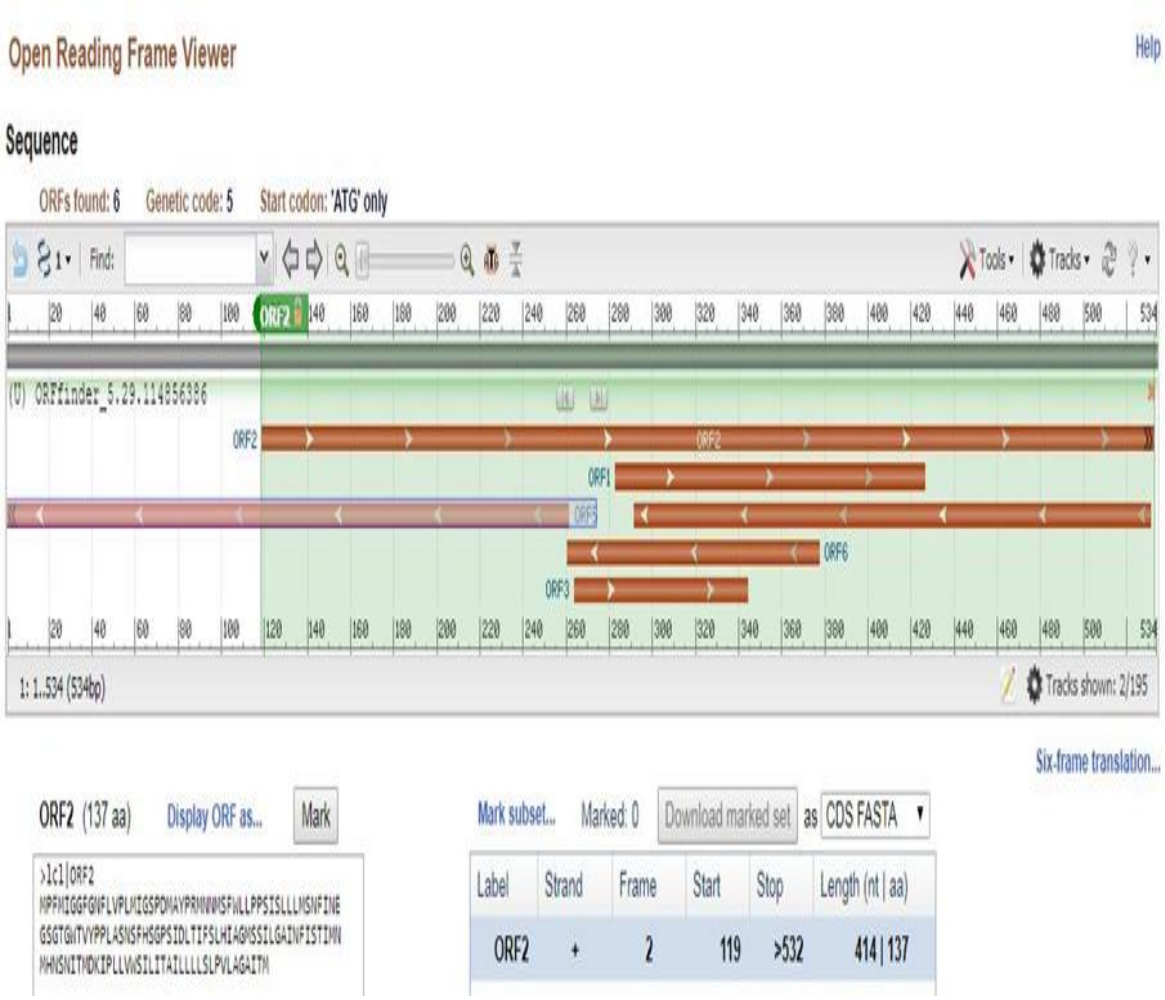

Fig:6.ORF2

The sequence was deposited in the Gen bank and accession ID was obtained (MK967472)

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LOCUS       Sample                  414 bp    DNA     linear   INV 23-MAY-2019
DEFINITION  Polyrhachis paracamponota mitochondrion.
ACCESSION   Sample
VERSION
KEYWORDS    .
SOURCE      mitochondrion Polyrhachis paracamponota
  ORGANISM  Polyrhachis paracamponota
            Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta;
            Pterygota; Neoptera; Holometabola; Hymenoptera; Apocrita; Aculeata;
            Formicoidea; Formicidae; Formicinae; Polyrhachis.
REFERENCE   1  (bases 1 to 414)
  AUTHORS   Venu,A. and Pvt. Ltd,O.L.
  TITLE     Phylogentics analysis of garden Ant
  JOURNAL   unpublished
REFERENCE   2  (bases 1 to 414)
  AUTHORS   Venu,A. and Pvt. Ltd,O.L.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-MAY-2019) Department of Zoology, Sacred Heart
            College, Thevara, Ernakulam, KERALA 682013, India
COMMENT     Bankit Comment: ALT EMAIL:beena.santhosh@gmail.com
            Bankit Comment: TOTAL # OF SEQS:1

            ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /db_xref="taxon:616396"
                     /country="India"
                     /collection_date="02-May-2019"
                     /collected by="Arunima Venu"

     gene            <1..>414
                     /gene="CO1"
                     /note="The CO1 gene can be used to identify individuals
                     belonging to the same species, as well as to distinguish
                     between individuals from different species"
     mRNA            <1..>414
                     /gene="CO1"
                     /product="cytochrome oxidase subunit 1"
     CDS             1..>414
                     /gene="CO1"
                     /codon_start=1
                     /transl_table=5
                     /product="cytochrome oxidase subunit 1"
                     /translation="MPFMIGGFGNFLVPLMIGSPDMAYPRMNNMSFWLLPPSISLLLM
                     SNFINEGSGTGWTVYPPLASNSFHSGPSIDLTIFSLHIAGMSSILGAINFISTIMNMH
                     NSNITMDKIPLLVWSILITAILLLLSLPVLAGAITM"
BASE COUNT      120 a     98 c     52 g    144 t
ORIGIN
        1 atgccattca taattggggg atttggaaat tttttagttc ctctaataat tggatctcct
       61 gatatagcat accctcgtat aaataacata agattctgac ttctaccccc ttcaatctcc
      121 ttactactta taagtaattt cattaatgaa ggatcgggga caggatgaac tgtttatccc
      181 cccctggcat caaactcatt ccacaggggc ccatcaattg atctaaccat tttttccctt
      241 catattgctg gaatatcatc aatcctaggg gctatcaatt ttatctccac tattataaat
      301 atacataact ctaacatcac tatagataaa atccccctat tagtatgatc tattctcatt
      361 acagctattc ttcttttact gtctctccca gtattagccg gagctattac cata
//
```

Fig.7: GenBank accession ID

DISCUSSION

Invertebrates are also likely to be powerful indicators of biodiversity responses to climate change, because of their high sensitivity to temperature and rainfall, and short generation times. Temperature changes, natural calamities and other demographic factors are responsible for migration of invertebrates like ants.

Regions with stable climates allow the evolution of finer specialisations and adaptations than do areas with variable climates [44,45]. As such, climatically stable areas tend to have high species richness and many range-restricted species [46].

Ants are an ideal focal taxon for studying species distributions and they are very sensitive to climatic variations. More than 12,000 different ant species have been identified. But while their ranks are large, only a small fraction of these species are considered home invading pests. Determining the types of ants in your home depends on a number of factors, including appearance, habitat and the region where you live. An ant's body can have one or two nodes, or "humps," on its thorax which helps identify the species. Species vary in both size and colour as well. Some ants can bite and some can sting, while others, such as the fire ant, can both bite and sting. The common ones that invade our homes are:

Acrobat ants (*Crematogaster*)

Worker ants are about 2.5 to 3 millimetres long with two nodes. Their colour varies from yellow-brown to brown and red-black to all black. They nest outside, primarily in moist wood and inside damaged wood structures, and sometimes in foam panel insulation. When threatened, worker ants of this species will lift their abdomens above their heads, resembling a scorpion. As they run with their abdomen held in the air, it gives the appearance that they are tumbling, hence the name, "acrobat ant." These types of ants are aggressive and will sting, although they are not frequent indoor invaders.

Argentine ants (*Linepithema humile*)

Worker ants are about 2.2 to 2.8 millimetres long and have one node. Their colour varies from light to dark brown. Outdoors, they nest in a variety of areas throughout the winter and in shadier spots during the summer. Indoors, they are sometimes found in wall insulation or voids. If stepped on, workers emit a musty smell. These ant types do not have the ability to sting.

Asian needle ant (*Pachycondyla chinensis*)

Worker ants are about 5 millimetres long and have one node. They are brown-black with dark orange mandibles, and mostly nest outdoors in shaded areas with high moisture. This species is less aggressive to neighbouring ant colonies than other ant types. It will sting if pressed against human skin or trapped in clothes. This happens most often during swarm season, which is between July and August. Its sting can be painful and sometimes causes a welt. A small number of cases where humans were stung resulted in anaphylactic shock.

Carpenter ants (*Camponotus*)

Multiple variations of this species exist across the United States. These types of ants rank high as structural pests. Due to such large variety, their physical description can be broad. The average length of workers is between 6 and 13 millimetres. Carpenter ants don't actually eat wood, but rather excavate it in order to create nests. The primary nest of these ants is typically outside, in trees or lumber. Indoors, they prefer roofs and woodwork near moisture. Homes in woodlands are at the most risk for structural damage. Some species are aggressive and will sting if their nest is disturbed.

Crazy ant (*Paratrechina longicornis*)

Workers are 2.2 to 3 millimetres long with one node. They are black-brown with extremely long legs and antennae. These types of ants are non-discriminatory nesters, forming colonies in moist or dry environments. Outdoors, they nest in plants, soil, heavy vegetation, mulch and garbage. Indoors, they can be found under carpets, in

wall voids and in houseplants. Crazy ants do not have stingers, but will move around sporadically if disturbed, thus earning their name. They are spread throughout the United States and are especially common along the Gulf Coast. Other common species in this subfamily include the Caribbean crazy ant (*Nylanderia pubens*) and the robust crazy ant (*Nylanderia bourbonica*).

Dark rover ants (*Brachymyrmex patagonicus*)

The average length of this ant is about 1.6 millimetres. They have one node for their body and are medium to dark brown in colour. They are commonly found in areas near people, and nest outdoors by grass edges, under objects on the ground and in parking lots. Indoor nests are sometimes found in bathrooms and kitchens. Dark rover ants have a preference for sweet liquids. Their large colony size can make them a nuisance, although they do not sting.

European fire ant (*Myrmica rubra*)

Workers are about 4 to 5 millimetres long. These ants have two nodes and can vary in colour, although most are a light reddish-brown. During warmer months, this species of ant forms nests in a wide range of outdoor habitats including gardens, lawns and shrubbery. They may form nests inside during cold months, particularly under bath tubs, water heaters or other warm areas with moisture. European fire ants are aggressive and can produce a painful sting when disturbed.

Field ant (*Formica*)

Workers vary in size from 4 to 8 millimetres long. They have one node and also come in a variety of colours including black, brown, yellow-brown and mixed red. This species of ant forms nests mostly in open areas of soil, dead wood or greenery. They are not commonly found indoors. Field ants have stingers and the ability to spray formic acid, which can induce a small amount of pain.

Many other ant species exist. They are largely considered pests because of their ability to produce large colonies, and their habit of foraging for food indoors. Some are more of a threat than others. Ants have the ability to transmit disease organisms through the contamination of food or sterile objects. Their stings can be painful and,

in rare circumstances, lead to anaphylactic shock. Carpenter ants also have the ability to cause damage to wood structures.

Ants such as *Polyrhachis* species, *Componotus* species look alike in our premises and we cannot distinguish them unless we don't observe their minute morphological characters.

Polyrhachis, are hyper diverse and distributed across the paleoartic tropics and temperate Australian region. Our biogeographic ancestral range analyses suggest that the evolution of Polyrhachis originated in South-East Asia, with an age of the modern crown-group Polyrhachis of 58 Ma. Spiny ants dispersed out of South-East Asia to Australia several times, but only once to mainland Africa around 26 Ma [47, 48]. They mimics spiders.

Camponotus is an extremely large and complex, globally distributed genus. At present, more than 1000 species and nearly 500 subspecies belonging to 45 subgenera are described and it could well be the largest ant genus of all. The enormous species richness, high levels of intraspecific and geographic variation and polymorphism render the taxonomy of Camponotus one of the most complex and difficult. Revisionary studies on Camponotus are generally confined to species groups and/or small geographical regions. These ants live in a variety of habitats and microhabitats and the sheer size of the genus makes any characterisation of their biology challenging. Nests are built in the ground, in rotten branches or twigs, or rarely into living wood and most species possess a highly generalistic diet. Antennal segment count 12 , Antennal club absent-gradual , Palp formula 6,4; 5,4 , Total dental count 4-9 , Spur formula 1 simple-barbulate, 1 simple-pectinate; 0, 0 ,Eyes present, Scrobes absent Caste polymorphic, Sting absent.(http://antwiki.orghttps://www.antwiki.org/wiki/Camponotus)

Our study aimed at molecular characterisation of an ant found in garden and we opted the mitochondrial genome region CO1 for determining the species and its phylogenetic analysis. The result showed that the ant captured from garden cannot be identified as any of the already reported ants yet. It shows only 89.66% similarity with the reported *Polyrhachis paracomponota* (JQ681070.1)with 99% coverage and

88.7% similarity with *Componotus parius* (KC685012.1) with the same coverage. More than 10% variation in the partial gene itself is observed and hence could not conclude this as a same species, but only a similar species with variations. Full genome sequencing of this species might come up with a new species that has not reported yet. Morphological similarities and behaviour could also be explored to identify this as a new species of ant.

Appendix

Fig.8: *Crematogaster*

Fig.9: *Linepithema humile*

Fig.10: *Pachycondyla chinesis*

Fig.11:*Camponotus*

Fig.12-*Paratechina longcornicus*

Fig.13: *Brachymyrmex patagicornis*

Fig.14: *Myrmica rubra*

Fig.15: *Formica*

Fig.16: *Polyrhachis paracamponota*

Fig.17: Sample

CONCULSION

The study conducted revealed that the ant species taken from garden, though similar to *Polyrhachis paracomponota* in morphological feature, it shows only 88.66% identity. Hence, we cannot confirm as same species but only as related species.

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EFFECT OF CASSAVA LEAVES ON THE GROWTH OF TILAPIA (*Oreochromis mossambicus*) AND ACCOMPANIED HISTOPATHOLOGICAL CHANGES IN ITS LIVER AND KIDNEY

DISSERTATION SUBMITTED TO MAHATMA GHANDHI UNIVERSITY IN PARTIAL FULFILLMENT OF THE POST GRADUATE DEGREE IN ZOOLOGY

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JUNE-2019

DEPARTMENT OF ZOOLOGY

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THEVARA

LARVICIDAL POTENCY OF *MYRISTICA FRAGRANS, TAGETES ERECTA AND CINNAMOMUM TAMALA* AQUEOUS LEAF EXTRACTSAGAINST *CULEX* SPECIES

Dissertation submitted in partial fulfillment of the requirements for the degree of

Master of Science in Zoology

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