Universal Journal of Agricultural Research 10(6): 761-784, 2022 http://www.hrpub.org
DOI: 10.13189/ujar.2022.100615

DNA Barcoding of Earthworms of Coorg Region of Karnataka, India and Study of Their Physio-Chemical Habitat

Lalitha Soumya[1,*], Kurian Raphael Poovathingal[2], G. Prakash Williams[3], Soonu Vadakke Kunnummal[4]

[1]Department of Biotechnology and Microbiology, Kannur University, Dr. Janaki Ammal Campus, Palayad, Thalassery, Kerala, India
[2]Head-Research & Development, TATA Coffee Limited, Pollibetta, Karnataka, India
[3]Department of Botany and Biotechnology, Bishop Moore College, Mavelikara, Alapuzha, Kerala, India
[4]Department of Biotechnology and Microbiology, Kannur University, Dr. Janaki Ammal Campus, Palayad, Thalassery, Kerala, India

Received November 9, 2021; Revised May 23, 2022; Accepted June 21, 2022

Cite This Paper in the Following Citation Styles

(a): *[1] Lalitha Soumya, Kurian Raphael Poovathingal, G. Prakash Williams, Soonu Vadakke Kunnummal , "DNA Barcoding of Earthworms of Coorg Region of Karnataka, India and Study of Their Physio-Chemical Habitat," Universal Journal of Agricultural Research, Vol. 10, No. 6, pp. 761 - 784, 2022. DOI: 10.13189/ujar.2022.100615.*

(b): *Lalitha Soumya, Kurian Raphael Poovathingal, G. Prakash Williams, Soonu Vadakke Kunnummal (2022). DNA Barcoding of Earthworms of Coorg Region of Karnataka, India and Study of Their Physio-Chemical Habitat. Universal Journal of Agricultural Research, 10(6), 761 - 784. DOI: 10.13189/ujar.2022.100615.*

Abstract Earthworms are considered as biological indicators of soil fertility and are the major macro fauna of soil. DNA tags are done to identify individuals belonging to the same species, as well as to distinguish between individuals from different species. In the present work, the earthworm fauna was investigated from three Taluks namely, Somvarpet, Virajpet and Madikeri of Coorg, a district situated in the south western part of Karnataka, India. This is the first scientific report on the earthworm species of Coorg and the physio-chemical properties of their soil. The physio-chemical characteristics of the soil were examined and DNA barcoding technology was used to identify the taxonomic status of earthworms. A total of 38 earthworm samples were collected, 30 of which were amplified using mitochondrial Cytochrome Oxidase subunit1 (CO1) gene specific primers. The sequencing data were deposited into Genbank and assigned to accession numbers (30 Accessions). The maximum likelihood technique was used to create the phylogenetic tree. Kimura's two-parameter model was used to calculate pairwise genetic distance. DNA barcoding results showed that Pontoscolex corethrurus was the most common earthworm species in Coorg. Progizzardus varadiamensis, Glyphidrilus annandalei, Amythas sp., Metaphire megascolidioides, Dichogaster bolaui and Acanthodrilidae sp were also observed. From the findings, it is observed that the mean pH of Coorg soil was found to be 6.2 with an electrical conductivity of 0.28 dS/m. Coorg soil contains a moderate level of Organic Carbon (O.C) content with an average of 1.74%. Thus, the study included extensive descriptions of earthworm species, as well as their DNA barcodes and the physio-chemical characteristics of their habitat, which help with future species identification.

Keywords DNA Barcoding, Earthworms, Cytochrome Oxidase Subunit1, Pontoscolex Corethrurus, Phylogenetic Tree, Pairwise Genetic Distance

1. Introduction

Earthworms, which are key ecosystem engineers, account for up to 90% of soil invertebrate biomass. In Oligochaetes, there are over 8,300 species of earthworms divided into 38 families and 811 genera [1]. Earthworms have the ability to biodegrade and bio transform chemical contaminants in their bodies, transforming them into less harmful compounds. Because earthworms have a tendency to attain large concentrations, they serve an essential role in soil ecology [2]. They are known as soil engineers and play an important role in soil food webs. They tilt and mix the

soil to enhance its structure and play a vital role in humus production. Earthworms are also crucial in enhancing the soil's water retaining capacity [3]. The distribution of earthworms is complicated, encompassing a wide range of biological differences [4]. Endemism is extremely common, both at the generic and species levels; 71 percent of genera and 89 percent of earthworm species are endemic [5].

The history of molecular research aimed at improving our understanding of earthworm taxonomy is relatively new. One of the major disadvantages of earthworm species, according to molecular ecologists, is their difficult classification [6]. In recent years, molecular techniques have shown to be a powerful and accurate tool that has proven to be extremely effective in overcoming the limits of traditional visual markers in documenting the current earthworm variety [7]. Molecular markers are widely acknowledged as excellent tools for classifying and examining plant and animal variety. The use of DNA barcoding has recently revealed an unanticipated number of species that are difficult to distinguish phenotypically or morphologically [8]. The international earthworm DNA barcoding initiative, which is centered at the Canadian Centre for DNA Barcoding, has shown a broad range of pedigrees among common earthworms [9]. The development of a reliable and quick approach for identifying species is a critical tool in the study of earthworms. Earthworm identification is exceedingly difficult and relies solely on diagnostic morphological characteristics [10]. DNA barcoding has evolved as a reliable tool for species identification, discovery, and biodiversity assessment in this technologically exciting day. It investigates the pattern of sequence divergence in a standardized gene area in order to speed up the identification and discovery of new species [11]. The variations discovered in the earthworm species were insufficient to distinguish their classes [12]. Molecular methods might help identify earthworm species, especially when morphological features are not taxonomically useful or are difficult to distinguish [13].

As a result, the research of their divergence is carried out using DNA barcoding analysis, which has shown to be a straightforward approach for identifying and classifying earthworm species [14]. The goal of DNA barcoding is to employ large-scale screening of one or a few reference genes to assign unknown people to species and to improve new species discovery. The creation of a comprehensive database of sequences, preferably linked with voucher specimens representing identified species, against which sequences from sampled people may be compared, is envisioned by proponents [15]. DNA barcoding has speed up the identification of specimens down to the species level, as well as the demarcation of species. While DNA barcoding may be used to match unknown specimens to recognized species, its utility for species delimitation is more contentious, as species discovery is dependent on current levels of haplotype diversity as well as the patterning of existing genetic variation within and across species [16].

In India, the Western Ghats and Eastern Himalaya areas have been designated as biodiversity hotspots due to the abundance of earthworm species. Despite covering just 2% of the world's territory, these locations are home to 10.5 percent of all known worldwide earthworm variety [17]. At the genus and species level, India has a relatively high percentage of endemic populations; around 71 percent of genera and 89 percent of earthworm species are local to the country. Apart from the endemic, a number of alien ambulant earthworm species have been discovered, which are now common in degraded ecosystems as a result of deforestation and intensive farming [18]. Indian earthworms are divided into three groups based on endemicity and dispersal: i) endemic or native species that are restricted to India; ii) exotic peregrine species that originated in other bio geographical regions; and iii) native peregrine species that evolved in India and now have widespread distribution in India and other bio geographical regions [19]. Hence it becomes necessary to adopt the DNA-based identification system as a valuable tool for identifying earthworm species and their physio chemical habitat.

The research presented here is structured into many sections, the first of which is the introduction. The second section discusses the literature review of academic papers. The technique and procedure of the work are explained in the third section. The fourth section contains the results of the work as well as the related discussions. The conclusion of the work is addressed in the last section.

2. Literature Survey

Tiwari et al [20], in their paper investigated the species richness of the study region; researchers employed a common barcode marker called cytochrome oxidase. Valid identification of voucher specimens was taken into account for characterization based on taxonomic relationships. Automatic Barcode Gap Discovery, operational taxonomic units, and network analysis were used to build the phylogenetic tree(s) using the best-fitted substitution model of evolution and species delimitation by Automatic Barcode Gap Discovery. The work demonstrated that the universality of a single locus remains idealistic, and that the utilization of additional common loci can help to improve earthworm diversity assessment. However, by utilizing a larger number of samples from various species and doing multiloci analysis, the study may be scaled up to a higher level. Furthermore, the proposed integrated approach to phylogenetic diversity can greatly improve conservation efforts aimed at maintaining a species' evolutionary potential.

Lone et al [21], in their research analyzed various earthworms, researchers examined earthworms gathered from reserve forests in Meghalaya, which is part of India's

North Eastern Region, a hotspot of biodiversity. To distinguish distinct species of the Kanchuria genus, they used integrative techniques incorporating morphoanatomical taxonomy and cytochrome oxidase 1 sequences utilizing DNA barcoding technology. Kanchuria daribokgrensis sp nov, Kanchuria karorensis sp nov, Kanchuria makhulensis sp nov, and Kanchuria mohiskulensis sp nov are the four new Kanchuria species discovered during the research. In addition, the Kanchuri turaensis and Kanchuri octotheca species were discovered. Six Kanchuria species have maximum intraspecific and minimum interspecific divergence of 6.11 percent and 14.85 percent, respectively. More wide geographic coverage, higher taxonomic variety, and study of bigger samples should all be included in future research.

Vabeiryureilai et al [22] in their work investigated some states in northeastern India and found they have set new records. Amynthas diffringens, A. gracilis, A. morrisi, and A. alexandri are new records from Nagaland; A. diffringens is a new record from Meghalaya; and A. hawayanus, A. incongruus, A. alexandri, and A. papulosus are new records from Mizoram. Many of the mtDNA CO1 sequences of Amynthas earthworms that we have documented were below 97 percent similarity when compared to BOLD systems and NCBI databases, in addition to the current record of 10 species. These findings also suggest that more species will be discovered at this location in the future.

Lalthanzara et al [23], in their research, investigated Earthworm specimens gathered from several habitats in Arunachal Pradesh, northeast India, using a random sample approach of digging and hand sorting. A traditional identification approach based on morpho-anatomical characteristics was used. Molecular characterisation began with the extraction of genomic DNA, followed by PCR amplification of the mtDNA CO1 gene. Sanger sequencing was performed on the PCR product using AB3500 Genetic Analyzers. A. gracilis was the most extensively dispersed species among the detected species, followed by A. corticis and Drawida nepalensis. With these seven new species, the total number of recognized earthworm species in Arunachal Pradesh now stands at 46. As a result, more research is needed, particularly in the huge protected regions with virgin forests.

Nouri-Aiin et al. [24], in their study provided a multiplex PCR approach that correctly scores the three species and is inexpensive in contrast to existing molecular techniques. In identifying species, multiplex PCR was as accurate as mitochondrial COI barcoding and better than morphological grading. The method uses different PCR fragments of varied lengths from the COI gene for each species. Amynthas agrestis, Amynthas tokioensis, and Metaphire hilgendorfi embryos were found in cocoons, juveniles, and adults of Amynthas agrestis, Amynthas tokioensis, and Metaphire hilgendorfi, respectively. According to comparisons of COI sequences (GenBank) with other populations of the same species and many other earthworm species, the primers invariably amplify just the three target species. When compared to standard COI barcoding, the multiplex PCR method is rapid and cheap. Furthermore, minute scratches of cells from living organisms may be instantly input into the PCR for easy identification, reducing costs by eliminating DNA extraction and allowing earthworms to be saved for ecological study. However, as further research this technique can also be customized for various earthworm communities..

For [20] by utilizing a larger number of samples from various species and doing multiloci analysis, the study may be scaled up to a higher level, for [21] More wide geographic coverage, higher taxonomic variety, and study of bigger samples should all be included in future research, for [22] more species has been discovered at that location in the future, for [23] more research is needed, particularly in the huge protected regions with virgin forests and for [24] further research in PCR molecular technique should be customized for various earthworm communities..Thus to have a virtuous benefit over other techniques, DNA barcoding and physio-chemical characteristics of the soil has to be implemented to determine the diverse taxonomy of earthworms. The sections below describe the study location and the process involved in the techniques used for identification.

3. Materials and Methods

This section explains the study location and the various methods performed in analyzing the samples.

3.1. Study Location

Coorg is located in Karnataka, India, between the latitudes of 11° 56' and 12° 56' N and the longitudes of 75° 22' and 76° 11' E. Coorg is Karnataka's smallest district, comprising 4102 square kilometers and accounting for 2.14 percent of the state's total land area. Forest covers approximately 33% of the district. Madikeri, Somvarpet, and Virajpet are the three Taluks in the district, having geographical areas of 1449 sq km, 999 sq km, and 1654 sq km, respectively. Madikeri, Coorg's district seat, is roughly 262 kilometers from Bangalore. Tandiondamol, at 1908 meters above mean sea level (MSL), is the district's highest mountain. With an annual rainfall of 160 inches and an average temperature of 21°C, Coorg is a cool and rainy district. The relative humidity is between 60 and 100 percent. Coorg district has been indicated in India map which is depicted in figure 1.

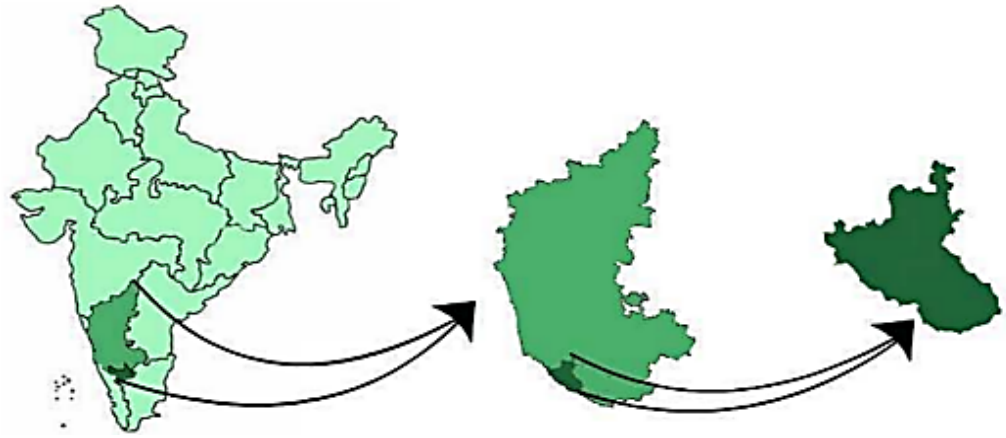

Figure 1. Map of India showing the Coorg district

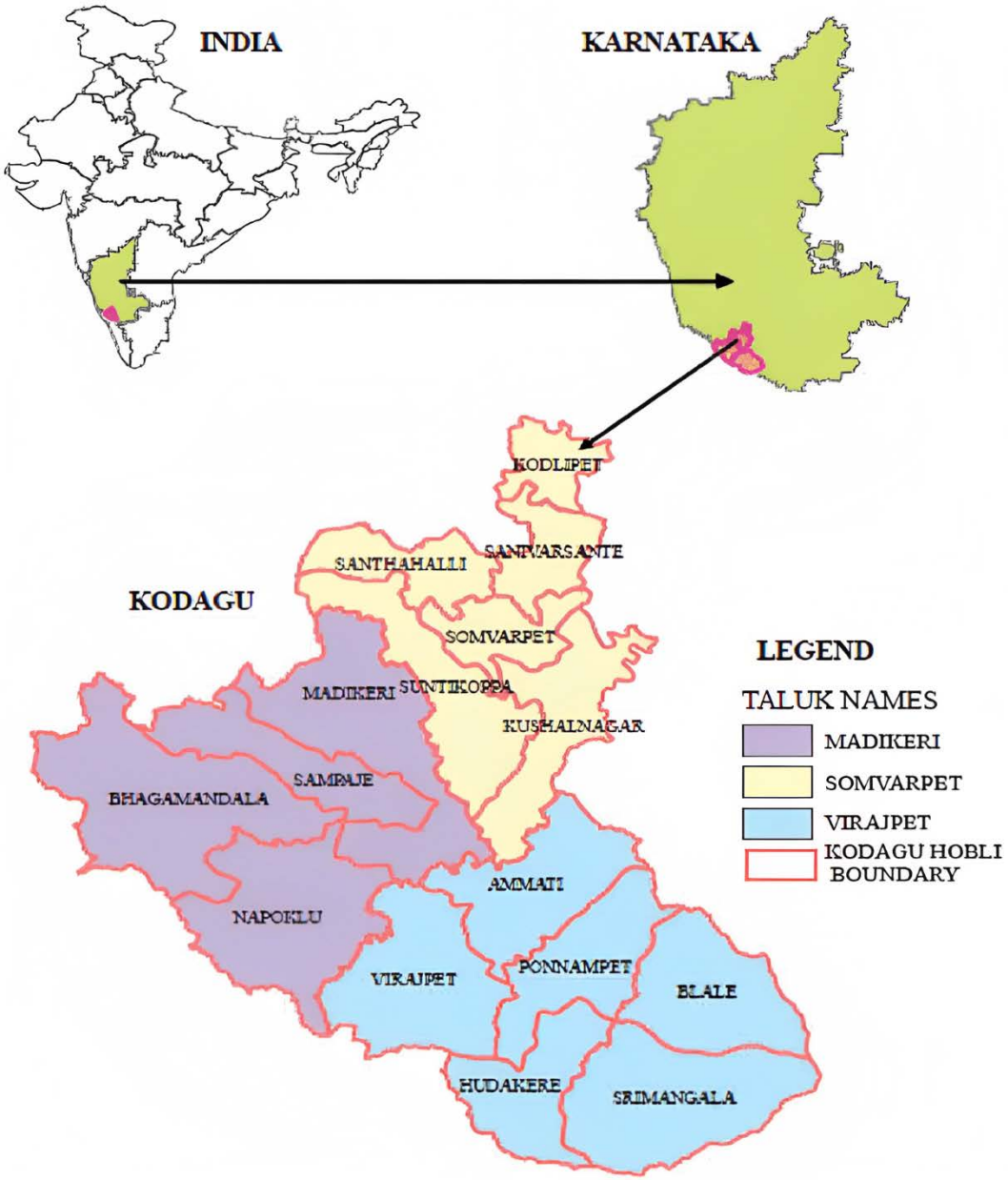

Figure 2. Map of Coorg showing the earthworm sampling spot

3.2. Sampling of Earthworm

Earthworms were collected from several locations of Coorg District in Karnataka as shown in figure 2. The sample locations were spread throughout three Taluks in Coorg. From September 2017 to January 2019, samples were collected. Earthworms were gathered from soil trenches ($25cm^2$ x 20cm depth) dug with a spade. The GPS (Global Positioning System) coordinates of the sampling sites were obtained and recorded. The earthworms were collected and cleaned in distilled water before being stored in 98 percent ethanol for subsequent analysis and molecular identification.

3.3. Sampling of Soil

To carry out the physio chemical analysis, 500 g of fresh soil was sampled from the pits at the time of earthworm sampling. Each sample were labeled carefully and kept aside for physio-chemical analysis.

3.4. Chemical Analysis

Using a multi-parameter tester 35 series (Thermo Fisher Scientific), the pH and Electrical Conductivity (E.C) of soil samples were measured. Walkey and Black fast titration technique and micro Kjeldhal method were used to calculate total organic carbon and total Kjeldhal nitrogen, respectively. The Bray and Krutz method and the flame emission technique were used to estimate available phosphorus and total potassium, respectively. Standard techniques were used to calculate the amount of exchangeable calcium and magnesium. Standard procedures were also used to identify heavy metals. All the determinations were carried out in triplicates.

3.5. Molecular Analysis

DNA was isolated from the posterior region of adult earthworm. DNA was electrophoresed in 1% agarose and seen under UV light as shown in figure 3.

Using particular primers, the barcoding region of the cytochrome oxidase gene was amplified. The master mix was used for PCR. 12.5 liters of master mix, 2 liters of DNA template, and 10 pmol of each primer were used in a 25 liter reaction mixture. For DNA amplification, a Mini Amp Plus thermocycler (Thermo, USA) was utilized. The reaction mixture was first incubated at 98 degrees Celsius for 3 minutes, followed by 35 cycles of amplification and a final extension at 72 degrees Celsius for 5 minutes. On a 2% agarose gel, PCR products were separated and bands were observed using a UV trans illuminator which is shown in figure 4.

Positive bands were purified according to the kit instructions using the QIA quick gel extraction kit (Qiagen TM, Germany). Following the manufacturer's directions, the cycling sequence was carried out with the Big Dye Terminator, version 3.1 kit. Using the appropriate primers, the sequence loaded plate was processed on an automated machine (3730 XL analyser). The Sequencher software 5.4.6 (Gene Codes Corporation) was used to modify and assemble sequence reads. Clustal W version 1.8 was used to align sequence similarities.

Figure 3. Total DNA extracted

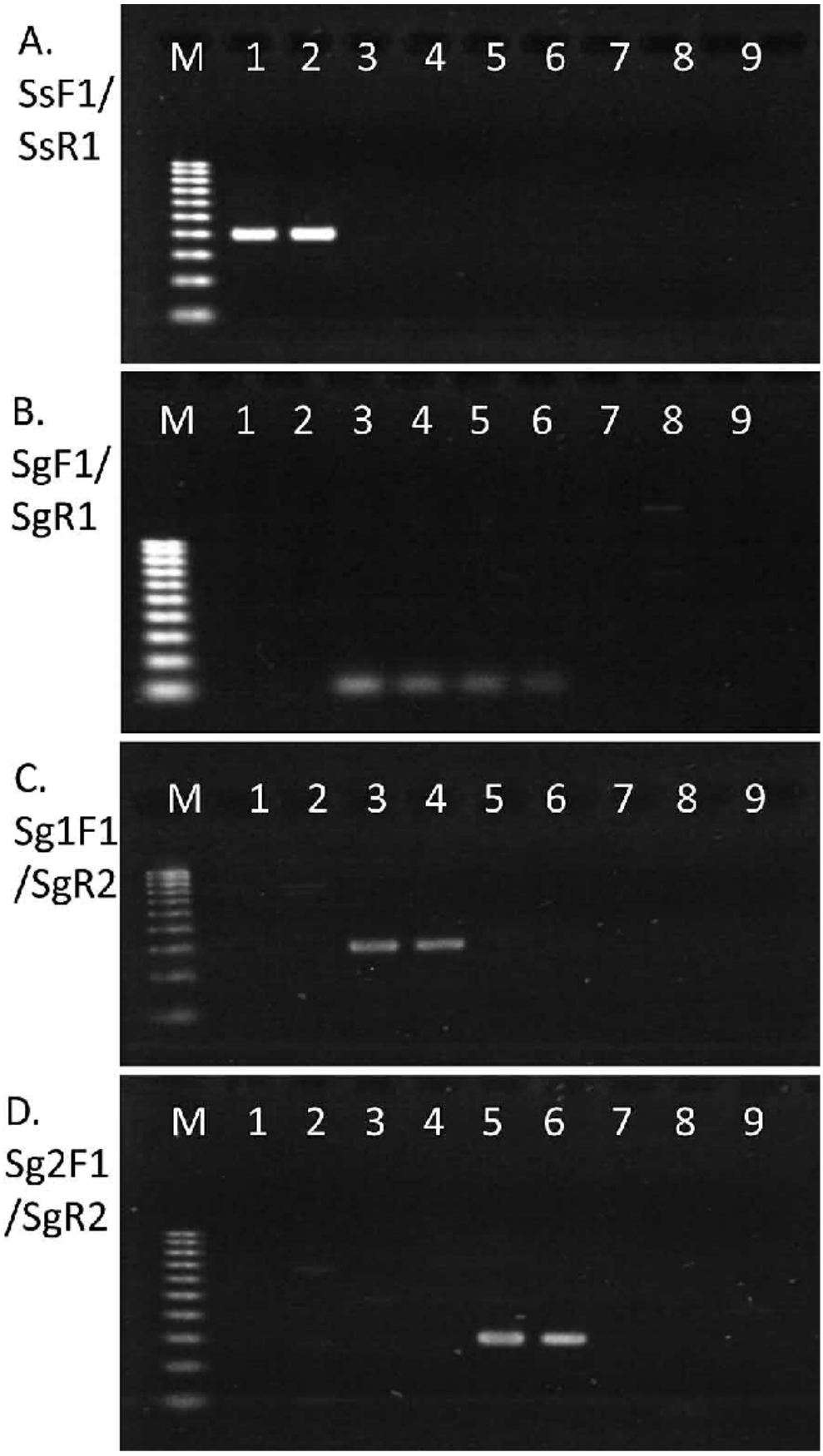

Figure 4. CO1 amplified gene product

Figure 5. Phylogenetic tree

The study of the evolutionary development of a species, a collection of organisms, or a specific trait of an organism is known as phylogenetic analysis. The phylogenetic study was carried out using MEGA X program. The maximum likelihood technique was used to create a phylogenetic tree for the cytochrome oxidase subunit 1 sequence which was depicted from figure 5. The branching confidence was assessed using 1000 bootstrap replications. Kimura's two-parameter model was used to calculate pairwise genetic distance.

3.6. Contribution to the Work

The main aim of the study is to use CO1 primers to DNA barcode the earthworms of Coorg district and evaluate the soil they inhabit in. The phylogenetic tree was created using maximum likelihood technique and Kimura's two-parameter model was used to calculate the pairwise genetic distance. Walkey and Black fast titration technique, micro Kjeldhal method, Bray and Krutz method and the flame emission technique were used to estimate the characteristics of the soil. The various results obtained in the identification of the earthworm species and the soil characteristics are discussed in the next section.

4. Results and Discussions

The data were analyzed in various locations and the results for the work are discussed in this chapter.

4.1. DNA Barcoding of Earthworm - Somvarpet Taluk

A total of 12 earthworm samples, as well as soil, were collected from various locations around the Somvarpet Taluk. The common earthworm species found in Somvarpet taluk were Pontoscolex corethrurus, Glyphidrilus annandalei, Amynthas alexandri, Amynthas hupeiensis, Progizzardus varadiamensis, and Dichogaster bolaui. A pH of 5.12 was preferred by Amynthas hupiensis obtained from Chettalli. The preferred pH of Amynthas alexandri, the sample taken from Hebbala, was determined to be 6.66. Glyphidrillus annandalei was collected from Nakoor Sirankala and Sunticoppa, and it thrived in a pH range of 5.31 to 7.51. In this soil, the NPK level and organic carbon content were determined to be satisfactory. Dichogaster bolaui was found in the Somvarpet Taluk with a pH of 5.8 and an organic carbon concentration of 2.03%.

4.1.1. Evaluation of the Physio-chemical Properties of the Soil Habitat of Earthworm - Somvarpet Taluk

Sample from Somvarpet Taluk

Sample Number 01: 7th Hoskote

The physio-chemical analysis of the sample 1 soil has been represented in table 1.

Table 1. Physio-chemical analysis of the soil (Sample 1)

7th Hoskote			Latitude			Longitude					
			12° 26'22.82°N			75°52'26.82°E					
pH	E.C	O.C	Clay	Slit	Sand	N	P	K			
	dS/m		%			(kg/ha)					
5.51	0.18	0.18	24.16	4	71.84	328.59	20.83	355.04			
Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
				(mg/kg soil)							
697.50	97.25	2.25	2.32	16.28	15.98	0.07	0.10	0.17	2.61	0.33	4.89

Genbank Accession: KU_BTMB_SJ01 (MN047288)
The blast annotation shows 91.73% identity with Progizzardus varadiamensis.

Sample Number 02: Chettalli

Table 2 depicts the physio-chemical analysis of the sample 2 soil.

Table 2. Physio-chemical analysis of the soil (Sample 2)

Chettalli			Latitude			Longitude					
			12° 19'37.58°N			75°49'20.29°E					
pH	E.C	O.C	Clay	Slit	Sand	N	P	K			
	dS/m		%			(kg/ha)					
5.12	0.19	0.22	20.16	6	73.84	201.39	17.85	388.64			
Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
				(mg/kg soil)							
762.50	76.75	6.46	8.90	11.42	34.08	0.10	0.10	0.16	1.20	0.27	5.20

Genbank Accession: KU_BTMB_SJ02 (MN047290)
The blast annotation shows 87.50% identity with **Amynthas hupeiensis**

Sample Number 03: Kushal Nagar

The physio-chemical analysis of the sample 3 soil has been shown in table 3.

Table 3. Physio-chemical analysis of the soil (Sample 3)

Kushal Nagar			Latitude			Longitude					
			12° 27'46.24°N			75°57'47.45°E					
pH	E.C	O.C	Clay	Slit	Sand	N	P	K			
	dS/m		%			(kg/ha)					
6.33	0.12	0.34	18.16	2	79.84	169.59	174.64	1018.08			
Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
				(mg/kg soil)							
1470.0	192	5.74	5.41	28.11	45.00	0.15	0.10	0.46	1.50	0.26	10.32

Genbank Accession: KU_BTMB_SJ03 (MN096840)
The blast annotation shows 100% identity with **Pontoscolex corethrurus**

Sample Number 15: Harangi

Table 4 depicts the physio-chemical analysis of the sample 15 soil.

Table 4. Physio-chemical analysis of the soil (Sample 15)

Harangi			Latitude			Longitude		
			12^0 25'24.03^{0}N			$75^0$44'17.11^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
7.68	0.33	1.13	14.16	6	79.84	211.99	475.98	1033.76
Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
2920	528.38	6.50	10.58	41.75	41.00	0.28	1.00	0.45	BDL	0.38	10.09

Genbank Accession: KU_BTMB_SJ15 (MN078213)
The blast annotation shows 100% identity with **Pontoscolex corethrurus**

Sample Number 17: Hebbale

Table 5 depicts the physio-chemical analysis of the sample 17 soil.

Table 5. Physio-chemical analysis of the soil (Sample 17)

Hebbale			Latitude			Longitude		
			12^0 30'43.34^{0}N			$75^0$57'53.05^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
6.66	0.48	1.78	28.16	4	67.84	222.59	582.49	908.32
Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
2240	437	7.15	25.28	124.75	206.75	0.41	1.10	0.60	0.57	0.38	37.42

Genbank Accession: KU_BTMB_SJ17 (MN060973)
The blast annotation shows 100% identity with **Amynthas alexandri**

Sample Number 18: Mallur

Table 6 depicts the physio-chemical analysis of the sample 18 soil.

Table 6. Physio-chemical analysis of the soil (Sample 18)

Mallur			Latitude			Longitude		
			12^0 38'26.14^{0}N			$75^0$57'26.18^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
7.29	0.42	1.72	12.16	6	81.84	180.19	128.51	515.20
Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
1760	990.88	29.0	27.58	173.25	30.00	0.35	0.80	0.23	BDL	0.32	39.03

Genbank Accession: KU_BTMB_SJ18 (MN060974)
The blast annotation shows 91.73% identity with **Progizzardus varadiamensis**.

Sample Number 19: Sanivarsanthe

Table 7 depicts the physio-chemical analysis of the sample 19 soil.

Table 7. Physio-chemical analysis of the soil (Sample 19)

Sanivarsanthe			Latitude			Longitude		
			12^0 43'47.59^{0}N			$75^0$53'9.49^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
5.76	0.03	1.03	26.16	2	71.84	190.79	4.66	357.28

Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
560	5.24	4.09	2.50	1.08	72.00	0.19	0.40	2.52	0.00	0.30	4.16

Genbank Accession: KU_BTMB_SJ19 (MN066318)
The blast annotation shows 91.85% identity with **Amynthas sp.**

Sample Number 21: Santhalli

Table 8 depicts the physio-chemical analysis of the sample 21 soil.

Table 8. Physio-chemical analysis of the soil (Sample 21)

Santhalli			Latitude			Longitude		
			12^0 37'33.65^{0}N			$75^0$48'39.52^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
6.88	0.39	2.44	12.16	6	81.84	180.19	74.12	472.64

Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
1200	3.07	7.16	32.20	281.00	131.75	2.00	2.80	0.69	BDL	0.35	49.35

Genbank Accession: KU_BTMB_SJ21 (MN066313)
The blast annotation shows 100% identity with **Pontoscolex corethrurus**

Sample Number 22: Nakoor Sirankala

Table 9 depicts the physio-chemical analysis of the sample 22 soil.

Table 9. Physio-chemical analysis of the soil (Sample 22)

Nakoor Sirankala			Latitude			Longitude		
			12^0 27'26.11^{0}N			$75^0$57'36.65^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
7.51	0.31	2.00	14.16	2	83.84	275.59	88.85	828.80

Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
3360	52.38	17.00	27.85	164.5	72.75	0.28	0.80	0.19	BDL	0.35	19.32

Genbank Accession: KU_BTMB_SJ22 (MN078212)
The blast annotation shows 100% identity with **Glyphidrilus annandalei**.

Sample Number 23: Sunticoppa

Table 10 depicts the physio-chemical analysis of the sample 23 soil.

Table 10. Physio-chemical analysis of the soil (Sample 23)

Sunticoppa			Latitude			Longitude		
			12^0 24'54.25^{0}N			$75^0$44'15.47^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
5.31	0.45	2.06	24.16	2	73.84	180.19	13.59	197.12

Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
1200	608.25	4.82	9.23	172.75	227.25	0.36	0.70	0.17	BDL	036	105.16

Genbank Accession: **KU_BTMB_SJ23 (MN066316)**
The blast annotation shows 99.63% identity with **Glyphidrilus annandalei**

Sample Number 24: Somvarpet

Table 11 depicts the physio-chemical analysis of the sample 24 soil.

Table 11. Physio-chemical analysis of the soil (Sample 24)

Somvarpet			Latitude			Longitude		
			12^0 31'11.17^{0}N			$75^0$50'28.67^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
5058	0.23	2.03	28.16	6	65.84	222.59	10.61	170.24

Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
1000	421.00	7.76	4.98	213.75	122.50	0.24	0.90	0.80	0.19	0.33	77.41

Genbank Accession: **KU_BTMB_SJ24 (MN004853)**
The blast annotation shows 89.65% identity with **Dichogaster bolaui**

Sample Number 25: Rangasamudra

Table 12 depicts the physio-chemical analysis of the sample 25 soil.

Table 12. Physio-chemical analysis of the soil (Sample 25)

Rangasamudra			Latitude			Longitude		
			12^0 26'36.36^{0}N			$75^0$56'2.27^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
6.64	0.21	1.41	26.16	4	69.84	190.79	19.66	304.64

Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
1000	280.50	6.35	3.20	61.50	53.00	0.20	0.50	0.36	0.00	0.30	15.67

Genbank Accession: **KU_BTMB_SJ25 (MN066315)**
The blast annotation shows 85.71% identity with **Glyphidrilus sp.**

Somvarpet Taluk soil has an average pH of 6.36, which was between the range of 5.12- 7.68. The percentage of clay, silt, and sand in the soil was determined to be 20.66 percent, 4.17 percent, and 75.17 percent, respectively. Within a range of 0.18-2.44 percent, the average O.C concentration was determined to be 1.36 percent. The average E.C was 0.28 dS/m, ranging from 0.03-0.48 dS/m. The soil had an average NPK concentration of 212.87 kg/ha, 134.32 kg/ha, and 545.81 kg/ha, respectively. The average calcium and magnesium concentrations were 1514.17 mg/kg and 37.73 mg/kg, respectively. On average, the sulphur level was found to be 31.50 mg/kg, whereas the boron concentration was determined to be between 0.26-0.38 mg/kg. The average amount of iron per kilogram was 107.51mg/kg. Zinc and manganese were found in average concentrations of 13.34 mg/kg and 87.67 mg/kg, respectively. Lead, chromium, nickel, and cadmium were also found in the soil, with average concentrations of 0.78 mg/kg, 0.87 mg/kg, 0.57 mg/kg, and 0.39 mg/kg, respectively.

4.2. DNA Barcoding of Earthworm - Virajpet Taluk

A total of 9 earthworm samples, as well as soil, were collected from various locations in Virajpet Taluk. The most common earthworm species in Virajpet Taluk was discovered to be Pontoscolex corethrurus. They were discovered in areas like Uddikeri, Virajpet, and Perumbadi. They favored a low to moderate O.C concentration and an acidic pH (5.34-6.96). (0.02-1.56 percent). The second most frequent species in Virajpet Taluk was Progizzardous varadiamensis. Acanthodrilidae sp., Amynthus hongyehensis, and Glyghidrilus annadalei were also discovered.

4.2.1. Evaluation of the Physio-chemical Properties of the Soil Habitat of Earthworm – Virajpet Taluk.

The evaluation of the soil sample in the study location is tabulated in tables below.

Sample Number 05: Uddikeri

Table 13 depicts the physio-chemical analysis of the sample 5 soil.

Table 13. Physio-chemical analysis of the soil (Sample 5)

Uddikeri			Latitude			Longitude		
			12^0 36'8.24^{0}N			$75^0$55'11.50^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
6.96	0.55	0.06	8.16	4	87.84	169.59	146.23	448.00
Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
1237.5	84	7.67	4	123.17	1544	0.18	0.30	0.15	2.82	0.29	45.19

Genbank Accession: **KU_BTMB_SJ05 (MN053022)**
The blast annotation shows 99.20% identity with **Pontoscolex corethrurus**

Sample Number 06: Ammathi

Table 14 depicts the physio-chemical analysis of the sample 6 soil.

Table 14. Physio-chemical analysis of the soil (Sample 6)

Ammathi			Latitude			Longitude		
			12^0 14'24.34^{0}N			$75^0$52'27.70^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
6.26	0.32	0.37	16.16	10	73.84	180.19	283.66	444.64
Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
1510	136.0	21.89	3.48	66.06	49.90	0.19	0.20	1.80	2.78	0.31	15.13

Genbank Accession: **KU_BTMB_SJ06 (MN078216)**
The blast annotation shows 81.67% identity with **Amynthas hongyehensis**

Sample Number 07: Virajpet

Table 15 depicts the physio-chemical analysis of the sample 7 soil.

Table 15. Physio-chemical analysis of the soil (Sample 7)

Virajpet			Latitude			Longitude		
			12^0 12'44.84^{0}N			$75^0$47'51.99^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
5.34	0.27	0.02	14.16	4	81.84	159.00	92.90	127.68

Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
320	30.50	14.88	3.14	29.52	23.26	0.04	0.10	0.06	2.03	0.27	5027

Genbank Accession: **KU_BTMB_SJ07 (MN004854)**
The blast annotation shows 100% identity with **Pontoscolex corethrurus**

Sample Number26: Siddapura

Table 16 depicts the physio-chemical analysis of the sample 26 soil.

Table 16. Physio-chemical analysis of the soil (Sample 26)

Siddapura			Latitude			Longitude		
			12^0 17'3.66^{0}N			$75^0$52'6.55^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
6.14	0.18	2.69	14.16	8	77.84	233.19	95.41	688.80

Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
600	276.13	5.25	8.45	36.50	133.25	0.32	1.10	0.37	0.01	28	7.81

Genbank Accession: **KU_BTMB_SJ26 (MN047289)**
The blast annotation shows 100% identity with Acanthodrilidae sp

Sample Number 31: Perumbadi

Table 17 depicts the physio-chemical analysis of the sample 31 soil.

Table 17. Physio-chemical analysis of the soil (Sample 31)

Perumbadi			Latitude			Longitude		
			12^0 19'22.90^{0}N			$76^0$2'43.59^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
5.57	0.09	1.56	12.16	4	83.84	222.59	147.81	212.80

Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
640	1.45	1.11	9.58	10.92	24.94	0.15	0.30	0.00	0.19	0.45	4.12

Genbank Accession: **KU_BTMB_SJ31 (MN078217)**
The blast annotation shows 100% identity with **Pontoscolex corethrurus**

Sample Number 35: Kakottuparamba

Table 18 depicts the physio-chemical analysis of the sample 35 soil.

Table 18. Physio-chemical analysis of the soil (Sample 35)

Kakottuparamba			Latitude			Longitude		
			12^0 14'20.99^{0}N			$75^0$46'44.40^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
5.58	0.23	2.03	28.16	6	65.84	222.59	10.61	170.24
Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
1000	421	7.76	4.98	213.75	122.50	0.24	0.90	0.80	0.19	0.33	77.41

Genbank Accession: **KU_BTMB_SJ35 (MK969111)**
The blast annotation shows 100% identity with **Glyphidrilus annandalei**.

Sample Number 36: Murnad

Table 19 depicts the physio-chemical analysis of the sample 36 soil.

Table 19. Physio-chemical analysis of the soil (Sample 36)

Murnad			Latitude			Longitude		
			12^0 15'20.35^{0}N			$75^0$46'28.73^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
5.91	0.19	2.09	10.16	2	87.84	222.59	72.80	246.40
Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
1320	262.75	2.01	10.95	25.25	210.50	0.23	0.80	0.12	0.11	0.37	5.66

Genbank Accession: **KU_BTMB_SJ36 (MK969110)**
The blast annotation shows 92.23% identity with **Progizzardus varadiamensis**

Sample Number 37: Heggala

Table 20 depicts the physio-chemical analysis of the sample 37 soil.

Table 20. Physio-chemical analysis of the soil (Sample 37)

Heggala			Latitude			Longitude		
			12^0 12'26.98^{0}N			$75^0$48'32.86^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
5.31	0.05	1.66	14.16	2	83.84	169.59	96.73	182.56
Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
440	1.90	1.20	1.54	16.11	28.36	0.08	0.30	.02	0.14	0.33	5.89

Genbank Accession: **KU_BTMB_SJ37 (MK969109)**
The blast annotation shows 91.73% identity with **Progizzardus varadiamensis**

Sample Number 38: Betoli

Table 21 depicts the physio-chemical analysis of the sample 38 soil.

Table 21. Physio-chemical analysis of the soil (Sample 38)

Betoli			Latitude			Longitude		
			12^0 21'19.67^{0}N			$75^0$44'52.94^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
5.53	0.17	1.97	18.16	10	71.84	296.79	42.91	425.60

Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
520	259.88	1.17	9.35	33	142	0.28	0.50	0.03	BDL	0.49	3.30

Genbank Accession: **KU_BTMB_SJ38 (MK969108)**
The blast annotation shows 99.20% identity with **Eudrilus eugeniae**

The average pH of the soil in Virajpet Taluk was 5.96, with a range of 5.34 to 6.96. The proportion of clay, silt, and sand in the soil was determined to be 15.05 percent, 5.56 percent, and 79.40 percent, respectively. Within a range of 0.02 -2.69 percent, the average O.C concentration was found to be 1.38 percent. The average E. C was 0.23 dS/m, ranging from 0.05 to 0.55 dS/m. The average NPK content of the soil was determined to be 208.46 kg per ha, 121.01 kg per ha, and 327.41 kg per ha, respectively. The average calcium and magnesium concentrations were 843.06 mg/kg and 163.73 mg/kg, respectively. The average sulphur level of the soil was 18.86 mg/kg, whereas boron content was determined to be between 0.27 and 0.49 mg/kg.

Iron content was 60.89 mg/kg on average. Zinc and manganese were found in average concentrations of 6.16 mg/kg and 253.30 mg/kg, respectively. Lead, chromium, nickel, and cadmium were also found in the soil, with average concentrations of 0.5 mg/kg, 1.15 mg/kg, 0.42 mg/kg, and 0.19 mg/kg, respectively.

4.3. DNA Barcoding of Earthworm - Madikeri Taluk

A total of 9 earthworm samples, as well as soil, were collected from various locations in Madikeri Taluk. Pontoscolex corethrurus was discovered to be the most common earthworm in Madikeri Taluk. Mekeri, Cherambane, Makanthoor, and Bettageri are the four locations where they may be found. Other species discovered were Amynthas sp., Metaphire megascolidioides, and Pontodrillus longissimus. Metaphire species were discovered to live in an acidic pH of 4.33. The soil's O.C concentration was found to be 3.13 percent. Despite the low NPK levels, Fe concentration was found as 636.50 mg/kg in the soil.

4.3.1. Evaluation of the Physio-chemical Properties of the Soil Habitat of Earthworm – Madikeri Taluk

The evaluation of the soil sample in the study location is tabulated in tables below.

Sample Number 04: Bagamandla

Table 22 depicts the physio-chemical analysis of the sample 4 soil.

Table 22. Physio-chemical analysis of the soil (Sample 4)

	Bagamandla			Latitude			Longitude		
				12^0 22'49.11^{0}N			$75^0$32'57.38^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K	
	dS/m		%			(kg/ha)			
6.64	0.26	2.88	16.16	6	77.84	307.39	500.88	578.68	
Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
3040	578.50	6.04	69.20	48.00	138	0.39	1.60	5.10	0.23	0.30	12.40

Genbank Accession: **KU_BTMB_SJ04 (MN091855)**
The blast annotation shows 100% identity with **Polypheretima elongate**

Sample Number 08: Kargunda

Table 23 depicts the physio-chemical analysis of the sample 8 soil.

Table 23. Physio-chemical analysis of the soil (Sample 8)

	Kargunda			Latitude			Longitude		
				12^0 20'56.45^{0}N			$75^0$38'7.52^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K	
	dS/m		%			(kg/ha)			
4.33	0.26	3.13	24.16	4	71.84	211.99	23.78	133.28	
Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
760	3.60	10.41	12.83	636.50	35.42	0.38	1.50	0.71	0.50	0.30	31.99

Genbank Accession: **KU_BTMB_SJ08 (MN066314)**
The blast annotation shows 87.10% identity with **Metaphire megascolidioides**

Sample Number 10: Mekeri

Table 24 depicts the physio-chemical analysis of the sample 10 soil.

Table 24. Physio-chemical analysis of the soil (Sample 10)

	Mekeri			Latitude			Longitude		
				12^0 24'8.92^{0}N			$75^0$44'22.28^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K	
	dS/m		%			(kg/ha)			
6.78	0.24	2.63	10.16	4	85.84	222.59	126.95	512.96	
Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
1200	5.72	10.41	33.98	291.50	223.50	0.38	2.00	0.24	0.49	0.35	47.19

Genbank Accession: **KU_BTMB_SJ10 (MN060972)**
The blast annotation shows 100% identity with **Pontoscolex corethrurus**

Sample Number 12: Cherambane

Table 25 depicts the physio-chemical analysis of the sample 12 soil.

Table 25. Physio-chemical analysis of the soil (Sample 12)

Cherambane			Latitude			Longitude		
			12^0 22'36.23^{0}N			$75^0$34'18.96^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
5.85	0.18	2.88	12.16	2	85.84	222.59	84.58	255.36
Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
				(mg/kg soil)							
2080	5.69	4.40	79.93	1.51	88.25	0.20	1.30	0.13	0.13	0.37	12.16

Genbank Accession: **KU_BTMB_SJ12 (MN078215)**
The blast annotation shows 100% identity with **Pontoscolex corethrurus**

Sample Number 13: Thalathumane

Table 26 depicts the physio-chemical analysis of the sample 13 soil.

Table 26. Physio-chemical analysis of the soil (Sample 13)

Thalathumane			Latitude			Longitude		
			12^0 25'27.04^{0}N			$75^0$46'55.64^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
7.25	0.75	2.03	14.16	4	81.84	254.39	176.40	873.60
Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
				(mg/kg soil)							
1480	893	15.00	25.98	148	49.75	0.29	0.90	0.16	BDL	0.36	71.02

Genbank Accession: **KU_BTMB_SJ13 (MN078214)**
The blast annotation shows 87.61% identity with **Amynthas sp**.

Sample Number 29: Makanthoor

Table 27 depicts the physio-chemical analysis of the sample 29 soil.

Table 27. Physio-chemical analysis of the soil (Sample 29)

Makanthoor			Latitude			Longitude		
			12^0 25'19.28^{0}N			$75^0$44'56.54^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
7.73	0.47	2.94	12.16	8	79.84	243.79	150.49	1568.00
Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
				(mg/kg soil)							
1640	224.50	18.00	21.45	223.00	68.75	0.36	0.90	0.16	BDL	0.51	81.61

Genbank Accession: **KU_BTMB_SJ29 (MN078211)**
The blast annotation shows 100% identity with **Pontoscolex corethrurus**.

Sample Number 30: Chettimane

Table 28 depicts the physio-chemical analysis of the sample 30 soil.

Table 28. Physio-chemical analysis of the soil (Sample 30)

Chettimane			Latitude			Longitude		
			12^0 22'47.38^{0}N			$75^0$34'25.63^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
5.41	0.10	2.94	12.16	2	85.84	243.79	23.37	184.80
Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
320	5.65	1.87	3.88	1.60	131.00	0.17	1.00	0.05	0.08	0.46	4.43

Genbank Accession: KU_BTMB_SJ30 (MN066317)
The blast annotation shows 92.98% identity with **Pontodrilus longissimus**

Sample Number 32: Kathelekadu

Table 29 depicts the physio-chemical analysis of the sample 32 soil.

Table 29. Physio-chemical analysis of the soil (Sample 32)

Kathelekadu			Latitude			Longitude		
			12^0 23'21.03^{0}N			$75^0$44'51.82^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
7.53	0.40	1.78	16.16	10.00	73.84	210.39	89.94	1100.96
Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
1920	5.23	7.25	7.33	58.25	62.50	0.27	1.40	0.05	BDL	0.32	54.24

Genbank Accession: **KU_BTMB_SJ32 (MN004852)**
The blast annotation shows 90.35% identity with **Pontodrilus longissimus**

Sample Number 34: Bettageri

Table 30 depicts the physio-chemical analysis of the sample 34 soil.

Table 30. Physio-chemical analysis of the soil (Sample 34)

Bettageri			Latitude			Longitude		
			12^0 18'39.35^{0}N			$75^0$40'41.44^{0}E		
pH	E.C	O.C	Clay	Slit	Sand	N	P	K
	dS/m		%			(kg/ha)		
5.02	0.28	1.13	16.16	2	81.84	180.19	62.35	107.52
Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Ni	Cr	B	S
(mg/kg soil)											
840	1.97	5.81	4.73	148.50	20.93	0.14	0.70	0.06	BDL	0.34	5.93

Genbank Accession: **KU_BTMB_SJ34 (MK969112)**
The blast annotation shows 100% identity with **Pontoscolex corethrurus**.

The average pH of Madikeri Taluk soil was determined to be 6.28, ranging from 4.33 to 7.73. The amount of clay, silt, and sand in the soil was determined to be 14.83 percent, 4.66 percent, and 80.51 percent, respectively, according to the physical characteristics of the soil. Within a range of 1.13-3.13 percent, the average O.C concentration was determined to be 2.48 percent. Within the range of 0.10-0.75 dS/m, the average E. C was 0.33 dS/m. The soil had an average NPK concentration of 207.28 kg/ha, 137.64 kg/ha, and 590.57 kg/ha, respectively. The average calcium and magnesium concentrations were 1475.56 mg/kg and 191.54 mg/kg, respectively. The average sulphur level of the Madikeri Taluk was 35.66 mg/kg, whereas the boron concentration was determined to be between 0.30-0.51 mg/kg. Iron content was 172.98 mg/kg on average. Zinc and manganese were found in average concentrations of 28.81 mg/kg and 90.9 mg/kg, respectively. Lead, chromium, nickel, and cadmium were all found in the soil, with average concentrations of 1.26 mg/kg, 0.29 mg/kg, 0.74 mg/kg, and 0.29 mg/kg, respectively.

5. Conclusions

In this research work, analysis of earthworm species and their physio chemical habitat was done in Coorg District consisting of three Taluks Somvarpet, Virajpet and Madikeri which showed the presence of predominant species in that area. Earthworm species were molecularly identified via DNA barcoding utilizing the cytochrome oxidase component. The unique sequence acquired from Cytochrome Oxidase CO1 subunit1 sequencing was deposited in Genbank and assigned accession numbers. The most prevalent earthworm species in Coorg was identified to be Pontoscolex corethrurus. Acanthodrilidae sp.,Progizzardus varadiamensis, Glyphidrilus annandalei, Amythas sp., Metaphire megascolidioides and Dichogaster bolaui. The physio-chemical analysis of the inhabiting soil had given the preferable natural habitat of the earthworm. It was determined that the pH of the soil was 6.2, with an E.C of 0.28 dS/m. NPK levels were found to be 209.54 kg/ha, 130.99 kg/ha, and 487.93 kg/ha, respectively. With an average of 1.74 percent, Coorg soil has a modest amount of O.C content. The proportion of clay, silt, and sand in the soil was determined to be 16.85 percent, 78.36 percent, and 4.8 percent, respectively. With 1277.60 mg/kg of soil, the calcium level of the soil was relatively high. Boron, sulphur, iron, and magnesium were found at concentrations of 0.35 mg/kg, 28.67 mg/kg, 113.79 mg/kg, and 221 mg/kg, respectively.

Appendix

The results of DNA barcoding of earthworm, the contig along with the species identified in the study locations were listed below. The accession numbers are quoted inside the brackets.

Samples from Somvarpet Taluk
Sample Number 1: 7th Hoskote

1. Genbank Accession: KU_BTMB_SJ01 (MN047288)

>TCAACTATACAACACTATCGTAACCGCACATGC
ATTTTTAATAATCTTCTTTCTTGTAATGCCAGTAT
TTATTGGTGGATTTGGAAACTGATTACTACCTTTA
ATACTAGGAGCACCAGACATAGCATTTCCACGAC
TAAACAACATAAGATTTTGACTACTTCCACCATC
CCTTATTCTACTAGTTTCATCCGCAGCGGTTGAAA
AAGGTGCCGGAACTGGATGAACAGTATATCCACC
CTTAGCAAGAAATATTGCACACGCTGGACCATCT
GTAGATCTTGCAATCTTCTCACTACATTTAGCTGG
TGCATCATCAATTTTAGGAGCAATCAACTTTATT
ACCACAGTAATTAACATACGATGATCAGGACTAC
GACTAGAACGAATCCCCCTATTTGTATGAGCCGT
AGTTATTACCGTAGTACTACTACTACTATCTTTAC
CTGTATTAGCTGGA GCCATTACCAT

The blast annotation shows 91.73% identity with **Progizzardus varadiamensis** with 7e-154 E- value and 84% query coverage according to the nucleotide homology

Sample number 2: Chettalli

2. Genbank Accession: KU_BTMB_SJ02 (MN047290)

>TTATTCTACTAGTTTCATCAGCAGCGGTTGAAAA
AGGTGCCGGAACTGGATGGACAGTATATCCACCC
TTAGCAAGAAACATTGCACACGCTGGACCATCTG
TAGATCTTGCAATCTTCTC
ACTACATTTAGCTGGTGCATCAT

The blast annotation shows 87.50% identity with **Amynthas hupeiensis** with 1e-37 E-value and 100% query coverage according to the nucleotide homology

Sample number 3: Kushal Nagar

3. Genbank Accession: KU_BTMB_SJ03 (MN096840)

>ATGAACTGTTTATCCACCCCTAGCAAGAAATAT
TGCTCATGCAGGTCCATCAGTGGACCTAGCCATC
TTCTCTCTTCACTTAACAGGTGCATCATCCATCTT
AGGAGCAATCAACTTCATTACCACAGTCATCAAT
ATACGATGAAATGGATTACGCTTAGAGCGAATCC
CACTTTTTGTATGGGCGGTAGTTATTACTGTAGTA
CTCCTCCTTCTGTCCCTACCCGTTCTTGCAGGAGC
TATTACAATACTACTAACAGATCGAAACCTTAA

The blast annotation shows 100% identity with **Pontoscolex corethrurus** with 4e-139 E-value and 100% query coverage according to the nucleotide homology.

Sample number 15: Harangi

4. GenbankAccession: KU_BTMB_SJ15(MN078213)

>ATGAACTGTTTATCCACCCCTAGCAAGAAATAT
TGCTCATGCAGGTCCATCAGTGGACCTAGCCATC
TTCTCTCTTCACTTAACAGGTGCATCATCCATCTT
AGGAGCAATCAACTTCATTACCACAGTCATCAAT
ATACGATGAAATGGATTACGCTTAGAGCGAATCC
CACTTTTTGTATGGGCGGTAGTTATTACTGTAGTA
CTCCTCCTTCTGTCCCTACCCGTTCTTGCAGGAGC

TATTACAATACTACTAACAGATCGAAACCTTAA

The blast annotation shows 100% identity with **Pontoscolex corethrurus** with 4e-139 E-value and 100% query coverage according to the nucleotide homology
Sample number 17: Hebbale

5. KU_BTMB_SJ17 (MN060973)

>TTATTCGAATCGAGCTAAGACAACCAGGCTCAT
TCCTAGGAAGTGATCAACTATATAACAC
GATTGTAACAGCCCATGCAT

The blast annotation shows 100% identity with **Amynthas alexandri** with 4e-33 E-value and 100% query coverage according to the nucleotide homology
Sample number 18: Mallur

6. Genbank Accession: KU_BTMB_SJ18 (MN060974)

>TCAACTATACAACACTATCGTAACCGCACATGC
ATTTTTAATAATCTTCTTTCTTGTAATGCCAGTAT
TTATTGGTGGATTTGGAAACTGATTACTACCTTTA
ATACTAGGAGCACCAGACATAGCATTTCCACGAC
TAAACAACATAAGATTTTGACTACTTCCACCATC
CCTTATTCTACTAGTTTCATCCGCAGCGGTTGAAA
AAGGTGCCGGAACTGGATGAACAGTATATCCACC
CTTAGCAAGAAATATTGCACACGCTGGACCATCT
GTAGATCTTGCAATCTTCTCACTACATTTAGCTGG
TGCATCATCAATTTTAGGAGCAATCAACTTTATT
ACCACAGTAATTAACATACGATGATCAGGACTAC
GACTAGAACGAATCCCCCTATTTGTATGAGCCGT
AGTTATTACCGTAGTACTACTAC
TACTATCTTTACCTGTATTAGCTGGAGCCATTACC
AT

The blast annotation shows 91.73% identity with **Progizzardus varadiamensis** with 7e-154 E-value and 84% query coverage according to the nucleotide homology
Sample number 19: Sanivarsanthe

7. KU_BTMB_SJ19 (MN066318)

>ATGAACAGTATATCCACCCTTAGCAAGAAATAT
TGCACACGCTGGACCATCTGTAGATCTTGCAATC
TTCTCACTACATTTAGCTGGTGCATCATCAATTTT
AGGAGCAATCAACTTTATTACC ACAGTAATTAA

The blast annotation shows 91.85% identity with **Amynthas sp.** with 2e-48 E-value and 100% query coverage according to the nucleotide homology
Sample number 21: Santhalli

8. KU_BTMB_SJ21 (MN066313)

>GGGGATTGCGCCTAGAACGAATTCCCCTCTTCG
TCTGAGCCGTAGTAATTACTGTGATCCTT
CTACTCTTATCGCTACCAGTATTAGCAGGAGCCA
T

The blast annotation shows 100% identity with **Pontoscolex corethrurus** with 8e-42 E-value and 100% query coverage according to the nucleotide homology
Sample number 22: Nakoor Sirankala

9. KU_BTMB_SJ22 (MN078212)

>TCACGCAGGCCCATCAGTAGACCTGGCCATTTT
TTCCCTGCACCTAGCAGGTGTTTCATCTATTTTAG
GTGCTATTAATTTTATTACGACAGTTGTAAATATA
CGCTGATCCGGACTTCGTATCGAACGAATTCCAT
TATTTGTATGATCTGTAGCAATTACTGTAGTTCTT
CTTTTATTATCACTACCAGTTCTTGCTGGGGCTAT
TACTATACTTCTAACCGATCGAAACAT

The blast annotation shows 100% identity with **Glyphidrilus annandalei** with 2e-117 E-value and 100% query coverage according to the nucleotide homology
Sample number 23: Sunticoppa

10. KU_BTMB_SJ23 (MN066316)

>TTACTTAGGAAGCGACCAATTATATAACACAAT
TGTTACAGCGCATGCCTTCATTATAATCTTTTTCC
TGGTAATGCCAGTATTTATTGGTGGCTTTGGAAA
TTGATTATTGCCTTTAATATTAGGCGCCCCAGATA
TAGCATTCCCACGACTCAATAACTTAAGATTTTG
ACTACTTCCCCCAGCTCTAATTCTCCTAGTATCCT
CTGCAGCTGTAGAAAAAGGAGCAGGCACTGGAT
GAACAGTATACCCACCTCTAGCAAGAAACAT

The blast annotation shows 99.63% identity with **Glyphidrilus annandalei** with 6e-140 E-value and 100% query coverage according to the nucleotide homology
Sample number 24: Somvarpet

11. KU_BTMB_SJ24 (MN004853)

The blast annotation shows 89.65% identity with **Dichogaster bolaui**

>ATGATTGGAGCTGGAATAAGCCTACTAATCCGA
ATCGAGTTGAGACAACCAGGAGCATTCCTTGGTA
GAGATCAACTATACAATACAATTGTAACGGCTCA
CGCATTTTTAATAATTTTCTTTTAGTTATACCAGT
ATTTATCGGGGGATTTGGAAATTGACTTCTACCT
CTAATACTAGGGGCCCAGATATAGCATTTCCACG
GCTAAATAACTTAAGTTTTTGACTACTACCGCCA
TCCCTAATTCTTCTAGTATCCTCTGCAGCAGTAGA
AAAAGGTGCTGGAACCGGATGAACAGTCTACCC
CCCATTAGCAAGAAACCTTGCACACGCGGGACCT
TCAGTAGATCTAGCAATTTTCTCCCTACACTTAGC
AGGTGCTTCTTCTATTCTAGGTGCAATCAACTTTA
TTACCACTGTTATTAATATACGATGATCTGGGCT
ACGATTAGAACGTATTCCACTATTTGTATGAGCA
GTAGTAATTACAGTAGTCTTATTACTTTTATCACT
ACCTGTTCTAGCAGGAGCAATTACCATATTATTA
ACAGATCGAAACTTAAACACT
TCATTTTTTGACCCTGCCGGAGGGGGAGACCCA

The blast annotation shows 89.65% identity with **Dichogaster bolaui** with 0 E-value and 99% query coverage according to the nucleotide homology
Sample number 25: Rangasamudra

12. KU_BTMB_SJ25 (MN066315)

>TTATTTAGGAAGCGACCAACTATACAACACAAT
TGTAACGGCACACGCCTTCATTATGATCTTTTTTT
TGGTAATACCAGTATTTATTGGGGGGTTCGGTAA
TTGATTACTACCTCTAATATTGGGGGCCCCAGAT
ATGGCATTCCCACGCCTCAA

The blast annotation shows 85.71% identity with **Glyphidrilus sp**. with 2e-34 E-value and 94% query coverage according to the nucleotide homology

Samples from Virajpet Taluk

Sample number 5: Uddikeri

13. KU_BTMB_SJ05 (MN053022)

>ATGATGCACCTGCTAGATGGAGGGAGAAGATG
GCCAGGTCTACTGAGGGCCCAGCGTGAGCAATAT
TTCTTGCTAGGGGGGGGTATACTGTTCATCCTGT
GCCGGCCCCCTTTTCAACGGCCGCGGACCTCACT
AGAAGGATTAAGGATGGCGGCAATAGTCAAAAT
CTTATGTTGTTTAGGCGAGGGAAGGCCATGTCTG
GCGCACCCAGTATTAGGGGGAGCAGTCAGTTGCC
GAA TCCCCCAATAA

The blast annotation shows 99.20% identity with **Pontoscolex corethrurus** with 2e-122 E- value and 100 % query coverage according to the nucleotide homology.

Sample number 6: Ammathi

14. KU_BTMB_SJ06 (MN078216)

>ATGGTTTTTTTCCTGGTAATACCTGTATTTATTG
GGGGGTTCGGAAACTGACTTCTGCCCCTGATACT
AGGTGCTCCAGACATAGCCTTCCCTCGTCTAAAC
AATCTAAGATTTTGGCTTCTCCCTCCTGCACTAAT
TTTACTAGTATCCTCTGCTGCTGTAGAAAAGGGG
GCCGGGTCAGGGTGAACCGTATATCCTCCTCTGG
CCAGGAATCTGGCCCATGCCGGGCCCTCAGTAGA
TCTAGCTATTTTTTCTCTTCATTTAGCGGGGGCCT
CATATATTCTGGGTTCCATCAACTTTATTACTACA
GTATTAACATACGATGACCTGGGATAAATGTAGA
ACGGATCCCCTTATTCGTATGAGGTGTAACTATT
ACAGTT

The blast annotation shows 81.67% identity with **Amynthas hongyehensis** with 5e-80 E-value and 96% query coverage according to the nucleotide homology

Sample number 7: Virajpet

15. KU_BTMB_SJ07 (MN004854)

>ATGGCCTTCCCTCGCCTAAACAACATAAGATTTT
GACTATTGCCGCCATCCTTAATCCTTCTAGTGAGG
TCCGCGGCCGTTGAAAAGGGGGCCGGCACAGGA
TGAACAGTATACCCCCCCCTAGCAAGAAATATCGC
TCACGCTGGGCCCTCAGTAGACCTGGCCATCTTCT
CCCTCCATCTAGCAGGTGCATCATCCATTTTAGGG
GCAATCAACTTCATCACTACAGTAGTGAACATACG
ATGAAGGGGATTGCGCCTAGAACGAATTCCCCTC
TTCGTCTGAGCCGTAGTAATTACTGTGATCCTTCTA
CTCTTATCGCTACCAGTATTAGCAGGAGCCATTACT
ATACTCTTAACAGACCGAAACCTAAACACCTCCTT
CTTTGACCCTGCGGGGGGGGGAGACCCTATCCTAT
ACCAACAT CTG

The blast annotation shows 100% identity with **Pontoscolex corethrurus** with 0.0 E-value and 100% query coverage according to the nucleotide homology.

Sample number 26: Siddapura

16. KU_BTMB_SJ26 (MN047289)

>TTAGATTCTGATTATTACCCCCATCACTAATTCT
CCTAGTATCCTCAGCTGCAGTAGAAAAAGGAGCG
GGTACAGGGTGAACTGTATATCCTCCATTAGCAA
GAAATTTGGCACATGCTGGACCATCAGTTGACCT
TGCAATTTTTTCCCTTCACTTAGCAGGTGCTTCAT

The blast annotation shows 100% identity with **Acanthodrilidae sp.** with 1e-82 E-value and 100% query coverage according to the nucleotide homology

Sample number 31: Perumbadi

17. KU_BTMB_SJ31 (MN078217)

>TTATTGGGGGATTCGGCAACTGACTGCTCCCCCT
AATACTGGGTGCCCCAGACATGGCCTTCCCTCGC
CTAAACAACATAAGATTTTGACTATTGCCGCCAT
CCTTAATCCTTCTAGTGAGGTCCGCGGCCGTTGA
AAAGGGGGCCGGCACAGGATGAACAGTATACCC
CCCCCTAGCAAGAAATATCGCTCACGCTGGGCCC
TCAGTAGACCTGGCCATCTTCTCCCTCCATCTAGC
AGGTGCATCAT

The blast annotation shows 100% identity with **Pontoscolex corethrurus** with 8e-126 E-value and 100% query coverage according to the nucleotide homology

Sample number 35: Kakottuparambu

18. KU_BTMB_SJ35 (MK969111)

>TTACTTAGGAAGCGACCAATTATATAACACAAT
TGTTACAGCGCATGCCTTCATTATAATCTTTTTCC
TGGTAATGCCAGTATTTATTGGTGGCTTTGGAAA
TTGATTATTGCCTTTAATATTAGGTGCCCCAGATA
TAGCATTCCCACGACTCAATAACTTAAGATTTTG
ACTACTTCCCCCAGCTCTAATTCTCCTAGTATCCT
CTGCAGCTGTAGAAAAAGGAGCAGGCACTGGAT
GAACAGTATACCCACCTCTAGCAAGAAACAT

The blast annotation shows 100% identity with **Glyphidrilus annandalei** with 2e-137 E-value and 100% query coverage according to the nucleotide homology

Sample number 36: Murnad

19. KU_BTMB_SJ36 (MK969110)

>TCAACTATACAACACTATCGTAACCGCACACGC
ATTTTTAATAATCTTCTTTCTTGTAATGCCAGTAT
TTATTGGTGGATTTGGAAACTGATTACTCCCCTTA
ATACTAGGAGCACCAGACATAGCATTCCCACGAC
TAAACAACATAAGATTTTGACTACTTCCACCCTC
TCTTATTCTACTAGTTTCATCAGCAGCGGTTGAAA
AAGGTGCCGGAACTGGATGAACAGTATATCCACC
CTTAGCAAGAAACATTGCACACGCTGGACCATCT
GTAGATCTTGCAATCTTCTCACTACATTTAGCTGG
TGCATCATCAATTTTAGGAGCAATCAACTTCATT
ACCACAGTAATTAACATACGATGATCAGGACTAC
GACTAGAACGAATCCCCCTATTTGTATGAGCCGT
AGTTATTACCGTAGTACTACTACTACTATCTCTAC
CTGTATTAGCTGGGGCCATTACCAT

The blast annotation shows 92.23% identity with **Progizzardus varadiamensis** with 3e-157 E-value and

84% query coverage according to the nucleotide homology
Sample number 37: Heggala

20. KU_BTMB_SJ37 (MK969109)

>TCAACTATACAACACTATCGTAACCGCACATGC
ATTTTTAATAATCTTCTTTCTTGTAATGCCAGTAT
TTATTGGTGGATTTGGAAACTGATTACTACCTTTA
ATACTAGGAGCACCAGACATAGCATTTCCACGAC
TAAACAACATAAGATTTTGACTACTTCCACCATC
CCTTATTCTACTAGTTTCATCCGCAGCGGTTGAAA
AAGGTGCCGGAACTGGATGAACAGTATATCCACC
CTTAGCAAGAAATATTGCACACGCTGGACCATCT
GTAGATCTTGCAATCTTCTCACTACATTTAGCTGG
TGCATCATCAATTTTAGGAGCAATCAACTTTATT
ACCACAGTAATTAACATACGATGATCAGGACTAC
GACTAGAACGAATCCCCCTATTTGTATGAGCCGT
AGTTATTACCGTAGTACTACTACTACTATCTTTAC
CTGTATTAGCTGGAGCCATTACCAT

The blast annotation shows 91.73% identity with **Progizzardus varadiamensis** with 7e-154 E-value and 84% query coverage according to the nucleotide homology.
Sample number 38: Betoli

21. KU_BTMB_SJ38 (MK969108)

>TTATTGGCGGATTTGGAAATTGATTACTTCCACT
AATACTGGGAGCGCCCGATATGGCATTCCCCCGA
CTAAATAACTTAAGATTTTGATTATTACCTCCTTC
ACTAATTCTCTTAGTTTCGTCAGCTGCAGTTGAAA
AGGGTGCAGGTACAGGATGAACTGTTTACCCGCC
ACTTGCAAGAAATCTTGCCCATGCCGGGCCCTCA
GTAGACCTAGCCATTTTCTCTCTTCATCTTGCAGG
GGCATCAT

The blast annotation shows 99.20% identity with **Eudrilus eugeniae** with 2e-122 E-value and 99% query coverage according to the nucleotide homology
Samples from Madikeri taluk
Sample number 4: Bagamandla

22. KU_BTMB_SJ04 (MN091855)

>ATGAACCGTATACCCGCCACTAGCAAGAAACAT
TGCACATGCTGGACCTTCAGTAGACCTAGCAATT
TTCTCTCTACACTTAGCAGGTGCCTCATCAATTCT
TGGGGCAATCAACTTTATTA CTACTGTCATTAA

The blast annotation shows 100% identity with **Polypheretima elongate** with 9e-63 E-value and 100% query coverage according to the nucleotide homology
Sample number 8: Kargunda

23. KU_BTMB_SJ08 (MN066314)

>TCAACTATATAATACCATCGTAACTGCGCACGC
ATTCATTATAATTTTCTTTTTAGTTATACCAGTAT
TTATTGGCGGGTTTGGAAACTGACTTCTACCCCTT
ATACTAGGAGCACCCGATATAGCATTTCCACGAC
TCAATAATATAAGATTTTGACTACTACCCCCGTCT
CTCATCCTTCTTGTCTCATCTGCAGCAGTAGAAA
AGGGAGCCGGTACAGGCTGAACAGTATACCCAC
CGCTAGCAA GCAA

The blast annotation shows 87.10% identity with **Metaphire megascolidioides** with 7e-72 E-value and 98% query coverage according to the nucleotide homology.
Sample number 10: Mekeri

24. KU_BTMB_SJ10 (MN060972)

>ATGAACAGTATACCCCCCCCTAGCAAGAAATAT
CGCTCACGCTGGGCCCTCAGTAGACCTGGCCATC
TTCTCCCTCCATCTAGCAGGTGCATCATCCATTTT
AGGGGCAATCAACTTCATCACTACAGTAGTGAAC
ATACGATGAAGGGGATTGCGCCTAGAACGAATTC
CCCTCTTCGTCTGAGCCGTAGTAATTACTGTGATC
CTTCTACTCTTATCGCTACCAGTATTAGCAGGAG
CCATTACTATACTCTTAACAGACCGAAACCTAAA
CACCTCCTTCTTTGA

The blast annotation shows 100% identity with **Pontoscolex corethrurus** with 2e-147 E-value and 100% query coverage according to the nucleotide homology
Sample number 12: Cherambane

25. KU_BTMB_SJ12 (MN078215)

>**ATGAACAGTATACCCCCCCCTAGCAAGAAAT
ATCGCTCACGCTGGGCCCTCAGTAGACCTGGC
CATCTTCTCCCTCCATCTAGCAGGGGCATCAT
CCATTTTAGGGGCAATCAACTTCATCACTACA
GTAGTGAACATACGATGAAGGGGATTGCGCCT
AGAACGAATTCCCCTCTTCGTCTGAGCCGTAG
T** AATTACTGA

The blast annotation shows 100% identity with **Pontoscolex corethrurus** with 1e-98 E-value and 99% query coverage according to the nucleotide homology.
Sample number 13: Thalathmane

26. KU_BTMB_SJ13 (MN078214)

>ATGAGCATTCCCACGACTAAACAACATAAGATT
TTGACTACTTCCACCCTCTCTTATTCTACTAGTTT
CATCAGCAGCGGTTGAAAAAGGTGCCGGAACTG
GATGGACAGTATATCCACCCTTAGCAAGAAACAT
TGCACACGCTGGACCATCTGTAGATCTTGCAATC
TTCTCACTACATTTAGCTGGTGCATCATCAATTTT
AGGAGCAATCAACTTCATTACCACAGTAATTAA

The blast annotation shows 87.61% identity with **Amynthas sp**. with 4e-69 E-value and 98% query coverage according to the nucleotide homology.
Sample number 29: Makanthoor

27. KU_BTMB_SJ29 (MN078211)

>ATGAACAGTATACCCCCCCCTAGCAAGAAATAT
CGCTCACGCTGGGCCCTCAGTAGACCTGGCCATC
TTCTCCCTCCATCTAGCAGGGGCATCATCCATTTT
AGGGGCAATCAACTTCATCACTACAGTAGTGAAC
ATACGATGAAGGGGATTGCGCCTAGAACGAATTC
CCCTCTTCGTCTGAGCCGTAGTAATTACTGTGATC
CTTCTACTCTTATCGCTACCAGTATTAGCAGGAG
CCATTACTAT

The blast annotation shows 100% identity with **Pontoscolex corethrurus** with 8e-126 E-value and 100% query coverage according to the nucleotide
Sample number 30: Chettimane

28. KU_BTMB_SJ30 (MN066317)

>ATGAACAGTATATCCACCCCTAGCAAGAAATAT
GGCACATGCAGGGCCATCAGTAGATTTAGCAATC
TTCTCTCTACATTTAGCCGGTGCATCATCAATTCT
TGGTGCCATTAA

The blast annotation shows 90.35% identity with **Pontodrilus longissimus** with 8e-33 E-value and 100% query coverage according to the nucleotide homology
Sample number 32: Kathelekadu

29. KU_BTMB_SJ32 (MN004852)

The blast annotation shows 90.35% identity with **Pontodrilus longissimus**

>ATGGCACATGCAGGGCCATCAGTAGATTTAGCA
ATCTTCTCTCTACATTTAGCCGGTGCATCATCAAT
TCTTGGTGCCATTAATTTTATCACTACAGTTATTA
ATATACGATGA
Sample number 34: Bettageri

30. KU_BTMB_SJ34 (MK969112)

>ATGAACAGTATACCCCCCCCTAGCAAGAAATAT
CGCTCACGCTGGGCCCTCAGTAGACCTGGCCATC
TTCTCCCTCCATCTAGCAGGTGCATCATCCATTTT
AGGGGCAATCAACTTCATCACTACAGTAGTGAAC
ATACGATGAAGGGGATTGCGCCTAGAACGAATTC
CCCTCTTCGTCTGAGCCGTAGTAATTACTGTGATC
CTTCTACTCTTATCGCTACCAGTATTAGCAGGAG
CCATTACTATACTCTTAACAGACCGAAACCTAAA
CACCTCCTTCTTTGA

The blast annotation shows 100% identity with **Pontoscolex corethrurus** with 2e-147 E-value and 100% query coverage according to the nucleotide homology

REFERENCES

[1] Kumar, Sathis, and P. Neelanarayanan, DNA barcoding of earthworms from lateritic semi evergreen forest of Kolli hill, a part of Eastern Ghats, Tamil Nadu, India, Research Journal of Biotechnology, Vol. 15, pp.11, 2020.

[2] Feledyn-Szewczyk, Beata, et al., Comparison of the effect of perennial energy crops and arable crops on earthworm populations, Agronomy, Vol. 9, No.11, pp. 675, 2019.

[3] Hallam, Jamal, and E. Mark Hodson, Impact of different earthworm ecotypes on water stable aggregates and soil water holding capacity, Biology and Fertility of Soils, Vol.56, No.5, pp. 607-617, 2020.

[4] Taheri, Shabnam, et al., Complex taxonomy of the 'brush tail'peregrine earthworm Pontoscolex corethrurus, Molecular Phylogenetics and Evolution, Vol.124, pp. 60-70, 2018.

[5] Narayanan, S. Prasanth, et al., First Record of Some Earthworm Species (Oligochaeta: Megadrile) from Kerala Part of Western Ghats Biodiversity Hotspot, Southwest India." National Academy Science Letters, Vol.42, No.6, pp. 509-512, 2019.

[6] Escudero, Gabriella Jorge, et al., Identification of earthworm species in Uruguay based on morphological and molecular methods, Agrociencia Uruguay, Vol. 23, No.1, pp. e58-e58, 2019.

[7] Marchán, Daniel Fernández, et al., Genome-informed integrative taxonomic description of three cryptic species in the earthworm genus Carpetania (Oligochaeta, Hormogastridae), Systematics and biodiversity, Vol. 18, No.3, pp. 203-215, 2020.

[8] Kumar, Mukesh, et al., Biochemical and molecular markers for characterization of chrysanthemum germplasm: A review, Journal of Pharmacognosy and Phytochemistry, Vol. 7, No.5, pp. 2641-2652, 2018.

[9] Keller, L. Erin, et al., Genetic diversity of an invasive earthworm, Lumbricus terrestris, at a long-term trading crossroad, the Champlain Valley of Vermont, USA, Biological Invasions, pp.1-13, 2020.

[10] Rice, A. Stanley, Stephen Karl, and A. Katherine Rice, The Polydora cornuta complex (Annelida: Polychaeta) contains populations that are reproductively isolated and genetically distinct, Invertebrate Biology, Vol.127, No.1, pp. 45-64, 2008.

[11] Dincă, Vlad, et al., Complete DNA barcode reference library for a country's butterfly fauna reveals high performance for temperate Europe, Proceedings of the Royal Society B: Biological Sciences, Vol.278, No.1704, pp. 347-355, 2011.

[12] Bottinelli, Nicolas, et al., An explicit definition of earthworm ecological categories–Marcel Bouché's triangle revisited, Geoderma, Vol.372, pp. 114361, 2020.

[13] Escudero, Gabriella Jorge, et al., Identification of earthworm species in Uruguay based on morphological and molecular methods, Agrociencia Uruguay, Vol. 23, No.1, pp. e58-e58, 2019.

[14] Maggia, Marie-Eugénie, et al., At each site its diversity: DNA barcoding reveals remarkable earthworm diversity in neotropical rainforests of French Guiana, Applied Soil Ecology, Vol.164, pp. 103932, 2021.

[15] Raju, K. Appala, Chapter-1 DNA Barcoding: A Novel Approach for Accurate Taxonomic Studies in Entomology, Recent Trends in, Vol.21, pp. 1, 2020.

[16] Phillips, D. Jarrett, J. Daniel Gillis, and H. Robert Hanner. Incomplete estimates of genetic diversity within species: Implications for DNA barcoding, Ecology and evolution, Vol.9, No.5, pp. 2996-3010, 2019.

[17] Mohan, Mahesh, M.S. Shylesh Chandran, and E.V. Ramasamy, Vulnerable Earthworm Species Identified from Nilgiri Biosphere, International Journal of Zoological Research, Vol.7, No.6, pp. 410-413, 2011.

[18] Yadav, Shweta, and Muner Mullah, A review on molecular markers as tools to study earthworm diversity, Int J Pure Appl Zool., Vol.5, pp.62-69, 2017.

[19] Ahmed, Shakoor, Jatinder Mohan Julka, and Hirdesh Kumar, Earthworms (Annelida: Clitellata: Megadrili) of Solan, a constituent of Himalayan Biodiversity Hotspot, India, Travaux du Muséum National d'Histoire Naturelle "Grigore Antipa, Vol.63, pp. 19, 2020.

[20] Tiwari, Nalini, et al., Interrogation of earthworm (Clitellata: Haplotaxida) taxonomy and the DNA sequence database, Journal of Asia-Pacific Biodiversity, Vol.14, No.1, pp. 40-52, 2021.

[21] Lone, Azhar Rashid, et al., Exploration of four new Kanchuria sp. of earthworms (Oligochaeta: Megascolecidae) from the North Eastern Region of India using DNA bar-coding approach, Journal of Asia-Pacific Biodiversity, Vol.13, No.2, pp. 268-281, 2020.

[22] M. Vabeiryureilai, et al., Study on the Amynthas (Kinberg, 1867) earthworm (Megascolecidae: Oligochaeta) diversity through DNA barcoding from Northeast India, Journal of Environmental Biology, Vol.41, No.4, pp. 867-873, 2020.

[23] H. Lalthanzara, et al., Diversity and New Records of Earthworms in Arunachal Pradesh, Northeast India, Journal of Environmental Biology, Vol.41, No.4, pp. 874-883, 2020.

[24] Nouri-Aiin, Maryam, et al., Identifying the unidentifiable: A PCR multiplex protocol for the diagnosis of invasive pheretimoid earthworm species, verified by morphological and barcode identification, Applied Soil Ecology, Vol.161, pp. 103822, 2021.