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ORIGINAL ARTICLE **OPEN ACCESS**

Karyological Studies of Few Species of Soil Engineers (Earthworms) through Ideokar, DNA Barcode: a New Technology

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ABSTRACT

Earth worms are ecosystem engineers are useful creatures in environment. Sources of bio indicators and markers. Objective, here is karyological analysis in taxonomic studies. Traditional method of morphological, anatomical and phylogenetical identification becomes inaccurate, Therefore study of chromosomes, numbers, the arrangements, length, centromere position, banding pattern are considered. karyological studies is carried out by Air drying method. Ideokar software is used to analyze Ideogram and karyotype, which is in the form of Digital Avenue in earthworms. DNA bar-coding is another method taken for complete identification of different species with different primers used in coding the species. (Utilizing short segment of mitochondrial COI) Result, the species are either too small or difficult to get complete set of chromosomes in meiotic slides but good number of mitotic slides was found. There by it is concluded that with centromere index, relative length the species are different and same is represented in the form of bar gram/histogram.

Key words: *Karyological studies, Earthworms, Ideokar, DNA Bar-coding, and centromere index.*

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INTRODUCTION

Earth worms belongs to Invertebrates, Annelid, oligochaete, These are soil engineers, harmless environmental soil creatures found in a wide range of climatic conditions. Playing role at chemical level, environmental recycling processes and genetically/molecular level. Sources of bio indicators, and markers. Karyological analysis is taken for taxonomical studies [1-5]. Method like, morphological, anatomical and phylogenetical identification becomes inaccurate, Therefore study of chromosomes, numbers, arrangements, length, karyrotype, banding pattern and centromere position presented in this studies. . Ideokar is a software used to study the earthworm karyotype digitally which represents chromosome number and size forming land mark of karyotype. It is a semi-automated type of karyotype analyzer which generates ideogram and this software is a user friendly, web interface helps to identify chromosome structure, genomes and genes. DNA bar-coding of earthworms is a successful biological/genetical tool in finalizing the taxonomical identification of these species at adult, young / juvenile phases. The method is utilizing a short segment of mitochondrial COI a genetic tag used in identifying and circumscribing, a tool in stream lining and identifying different species.DNA barcode and coding is a potential solution to karyotyping of any organisms [6-9]. Extensive routine for identifications it provides quick and affordable challenges from the previous methodology.

Objectives of the Study

1. Karyotype of earthworms to study diversity of evolution, cytology and taxonomy of few species of earthworms.
2. Evolutionary, cytology and cyto systematic.
3. Karyotype and Ideogram of earthworms using Ideokar software.
4. DNA bar coding to classify the organisms at sub species at sub species level.
5. High banding resolution, relative length, length of short/long arms and centromere Index.

MATERIAL AND METHODS

An elaborate field work is carried out to collect different species from different places like crop fields, municipality, near bore wells depending upon the availability of these different species of earthworms are collected in rainy season. The soil in the ground is dug, worms are with care and gently handpicked and put these earthworms into container containing native soil reared them to get eggs, young ones and

adults. For further research work and standardization is carried after many trials. Karyology. Collection, preservation and identifications are carried out as per standard procedure.

1. Cellophane method.
2. Squash method.
3. Air dry method.

Air drying method was more suitable technique which I found in this studies. Air drying technique according to and Venkatachalaih and Chowdaih [10] this method is adapted to preparing metaphase chromosomes. This technique is adopted as it is most suitable/reliable procedure in extracting the chromosomes. Metaphase Chromosomes spread was prepared in fully grown adult earthworms.

METHOD 1

Cellophane method was not suitable for animal tissue, it is employed only in plant tissues**.**

METHOD:2

SLIDE PREPARATION

Tail end after colchicines treatment is mixed with 3:1 mixture of ethanol and glacial acetic acid at 4 degree Celsius [1] passed on to various different grades of alcohol and dropped on to the slide later dried with blow drier

This colchicines solution was injected into anterior region between head and clitellum in the ratio of 0.1: 1 that is 1ml for 1gm of body weight, and kept for one day/24 hrs. After one day, the earthworms were removed, now they are used for dissecting the organisms. Dissection is carried out by cutting on the dorsal surface of earthworm. The internal structural like female reproductive organs(ovaries), testis and seminal vesicles are being removed to prepare the slides. Meiotic slides are done by taking reproductive organs and mitotic slides are prepared by taking gut and tail parts/ regions.

Staining is done by using Giemsa stain for nearly 30-45 minutes, later stain is removed, add one or two drops of glycerin over the material, cover the material with cover slip, seal with DPX. Slide is now ready to view under microscope,

METHOD: 3:

SLIDE PREPARATION:

1. Take dissected material (gonads and tissue of tail region) in a cavity block.
2. Hypotonic solution is prepared by adding 0.56 gms of KCl powder into 100ml of distilled water
3. Add 5ml of hypotonic solution to the material taken in a cavity block.
4. Mince the material thoroughly so as to make homogenized solution.
5. Mitotic preparation was taken from gut and tail region and Meiotic slides done by taking from ovaries and testis were carried out by subjecting these tissue into 0.065% to 0.075% , hypotonic potassium chloride solution later this is made to settle down say for about 1hr to 1 to 1.30 hours. The tissue later were treated with corney's fixative is prepared freshly (3;1)

Methyl alcohol three parts and 1part glacial acetic acid)and allowed to settle down nearly for about 20-30 minutes. Changes are given for about 3-4 times. Whenever slides are prepared always fresh fixative is taken. Later the method: 3. technique according to *a*nd Venkatachalaih and Chowdaih [10] with little modification is adapted. The slides were stained , Acetoorcienor Giemsa stain is used later rinsing is done with freshly prepared distilled water. Later it is subjected to air drying technique is carried out,

Three species taken for these studies are *Eudriluseuginea, Eisenia fetida* and *Polypheritimaelongate.*The slide were prepared for chromosome studies. Metaphase plates was traced at different focusin both mitosis/ meiosis divisions, eight each focus in mitosis/meiosis were taken.. Morphometric analysis and karyotype of different species like P*olypheretima elongate, Esienia fetida and Eudrilus eugeniae* and was carried. Below is the results of chromosomal analysis.Morphological studies and cytological studies respectively in the following below table:

Calculation of Centromere index (CI):-

$$\text{Centromere index} = \frac{\text{Length of short arm} \times 100}{\text{Whole length of chromosome } \{S = (l+S)\}}$$

Centromere index is measurement from chromosome images taken from the ideokar image cytometry.

Calculation of Arm ratio:-

It is the length of the longer arm of the chromosome divided by the length of the shorter arm. Arm ratio(AR) = $\frac{\text{Length of long arm}}{\text{Length of short arm}}$

$$\text{Relative length} = \frac{}{\text{Length of whole chromosomes X 100}}$$

Total length of all the chromosomes in the haploid set including one being measured.

RESULTS AND DISCUSSION

The differences in genera/species reveal greater similarities in homologous chromosome. The structure and shape of the chromosomes are very clearly seen under metaphase spread. As many different types of chromosomes seen, among them only Meta centric, sub metacentric and telocentric chromosomes were seen but germinal centromeres were not observed. The examined slides showed some chromosomes which were rod shaped in mitotic plates, chromosome appeared in metaphase stage was very clear, but the prophase stage the chromosomes were short and they appeared as short arms again it was not so very clear. The theory of splitting or Fusion was not applicable for this basic principle, therefore it becomes difficult to explain the structures of chromosomes and centromere. The basic chromosome numbers, structure, position, length etc therefore was measured by using a new technology Ideokar. Relative length, chromosome pair numbered in haploid state are measured using Ideokar.. Many times it is noted that the accuracy of obtaining the relative length was becoming difficult. The chromosome arm length p and q arms also very difficult to observe during that time only gross differences are taken for studies. The gross differences in morphology structure and chromosome number are taken into consideration. Even the differences in male and female chromosomes and size differences were very clear cut. Oogenesis, meiotic slides when compared with spermatogenesis, the chromosomes of gut and tail regions were very prominent, clear and accurate. The chaisma frequency though difficult to trace but some of the slide showed few chaisma frequency. When three species were compared all the three species revealed all most same. The only group of organisms, which could show differences, is the genus the native earthworms is *Pheretima*, it is concluded that the youngest shoot may be the *Megascolicidae. E.foetida* species is one such species having ornamental setae. When compared with other species, this has high degree of reproduction rate and also the development of an embryo. All support the extensive application in vermicompost.

Karyotype and Ideogram of *Eisenia foetida* species.

Centromere index was calculated we found that all the haploid chromosomes morphology/ length areshown in the following table. Another Histogram is also made to compare the morphology/length of the chromosomes and centromere of the chromosoms of all the three species. Centromere index is also calculated. These are the type of chromosomes were seen,

V-shaped chromosomes

J= Large J shaped chromosomes with arm ratio little lesser than 0.8

I= Medium sized I shaped chromosomes

L = Medium sized L shaped chromosomes.

The Karyotype of *Eisenia fetida* reveals that the presence of Chromosomes numbers N (haploidy)=11 and 2N (diploidy) =22.

The chromosomesare classified on the bases of morphology/ position of the centromere.

1) Metacentric :-Centromere- Position is in the centre.

2) Sub-Metacentric –Position of Centromere is slightly away from the centre.

3) Sub-Telocentric –Position of Centromere is somewhere at sub-terminal position in the chromosome.

4) Telocentric - Centromere is present at the terminal end.

5) Acrocentric - Centromere is present at the end,

Small arm is so small that it is very difficult to see not clearly visible.

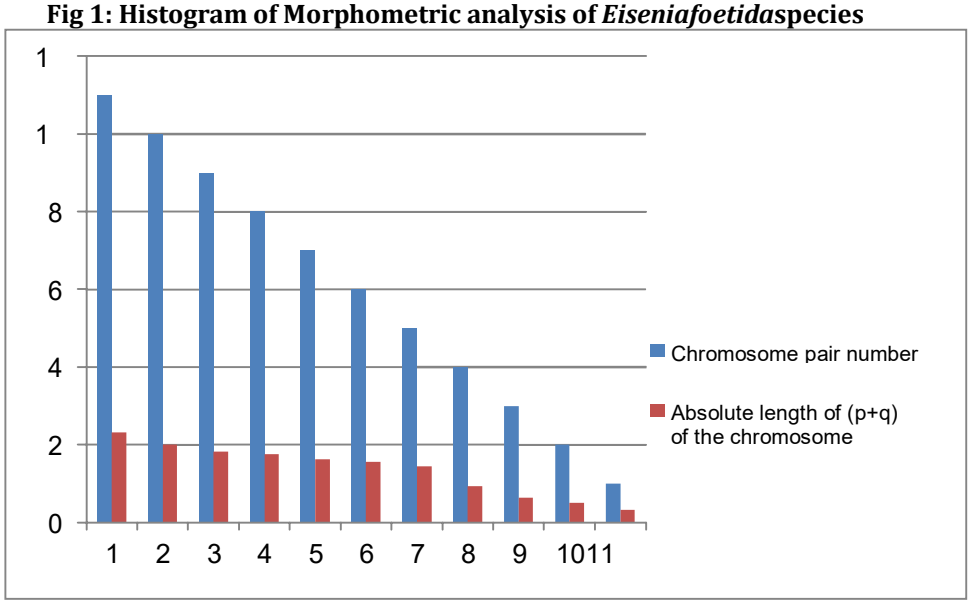
Table: 1. Karyotype and Idiogram. Species number 01**.** *Eisenia foetida.*

Chromosome pair number	Absolute length(p+q) Of the chromosomes.
1	2.32
2	2.00
3	1.83
4	1.76
5	1.63
6	1.56
7	1.45
8	0.94
9	0..64
10	0.51
11	0.33

Histogram/karyology (karyotype and Idiogram):-

Showing the chromosomes in the order of decreasing in chromosome pair numbers/length from forst to last the eleventh.

Fig 1: Histogram of Morphometric analysis of *Eiseniafoetida*species



ANALYSIS OF THE KARYOTYPE MORPHOMETRICALLY OF *EISENIA FOETIDA*
N (HAPLOID)=11, 2N (DIPLOID)=22)

Chromosome pair no.	mean Length in um of short arm(p)	mean Length in um of long arm (q)	Absolute Length (p+q) of chromosome	Arm ratio q/p	Total complement Length %	Centromeric Index	Nomenclature
1	0.86	1.46	2.32	1.69	7.68	37.06	Submetacentric
2	0.64	1.36	2.00	2.12	6.62	32.00	Submetacentric
3	0.66	1.10	1.76	1.66	5.83	37.50	Submetacentric
4	0.61	1.22	1.83	2.00	6.06	33.33	Metacentric
5	0.48	1.08	1.56	2.25	5.16	30.76	Metacentric
6	0.22	0.72	0.94	3.27	3.11	23.40	Subtelocentric
7	0.41	1.04	1.45	2.53	4.80	28.27	Subtelocentric
8	0.39	1.24	1.63	3.17	5.41	23.92	Subtelocentric
9	0.08	0.54	0.64	6.75	2.12	12.50	Telocentric
10	0.05	0.46	0.51	9.20	1.68	09.80	Telocentric
11	0.02	0.31	0.33	0.15	1.09	06.06	Acrocentric

Table 2: ANALYSIS OF THE KARYOTYPE MORPHOMETRICALLY OF *EISENIA FOETIDA*

MORPHOMETRIC ANALYSISOF CHROMOSOMES.

Fig 2: *Eiseniafetida. (N (SINGLE SET)=11, 2N (DOUBLE SET)=22)*

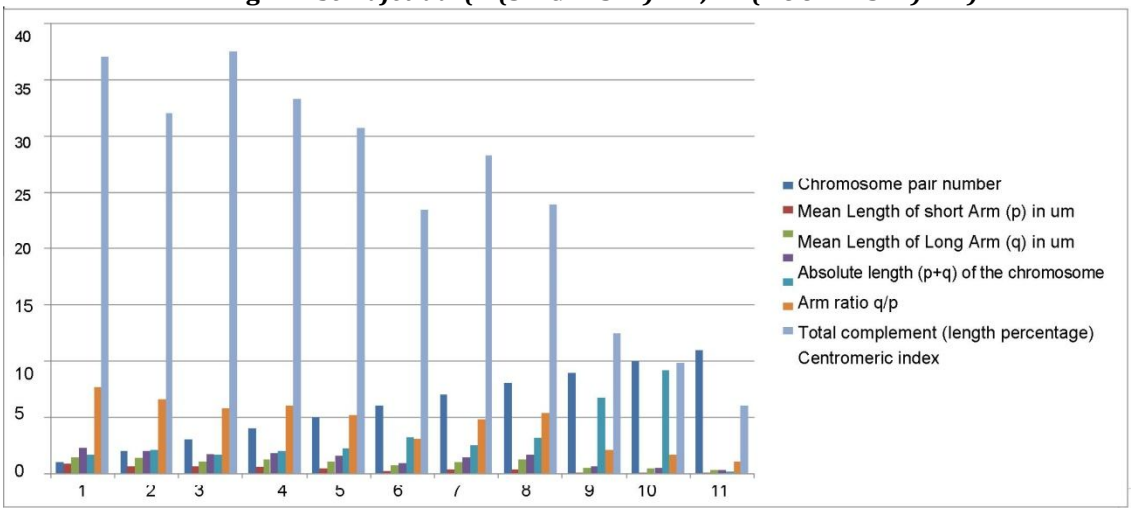


Fig 3: Chromosomes viewed under Microscope

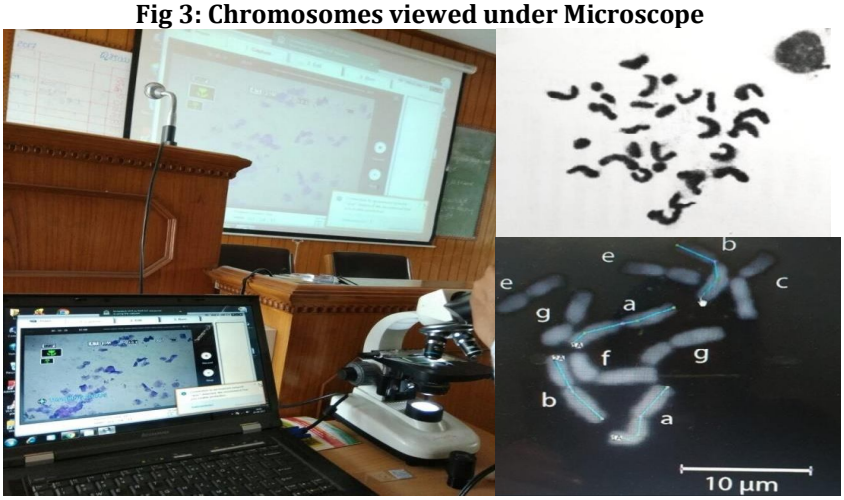


Fig 4: DNA barcode of *Eisenia foetida*

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