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Full Length Research Article

New Record, Distribution, Genetic Diversity of Vermiresources and Composting Potential of Local Earthworm Species from Northern Region of India

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Abstract

The present work is an update on earthworm fauna of Eastern Haryana forming main part of Trans-Gangetic region. Survey of earthworm species in this Eastern Haryana with reference to diversity, distribution, and composting efficiency of local species revealed the presence of 14 species of earthworms belonging to 5 families, 11 genera. Out of 14 species Two species belonging to family Ocnerodrillidae (*Gordiodrilus elegans*) and Lumbricidae (*Eisenia andrei*) was reported for the first time from Trans-Gangetic region of Haryana. During the survey *Lampito mauritii* was the dominant species in this surveyed region representing 54.81% of total earthworm population. This anecic species had been recorded almost from all studied pedoecosystems. Therefore, the potential efficiency of this species of earthworms (*L. mauritii*) has been done by culturing them in kitchen waste rich organic matter. It was found that *L. mauritii* can be used as a vermicompost species in the northern region of India. Along with this the genetic diversity of some species had also been studied and phylogenetic relatedness among different species of earthworms was constructed which showed the presence of highly diverged lineages species of earthworms inhabiting Haryana soils. For genetic studies 10 random primers (OPA-1, OPA-2, OPA-3, OPA-4, OPA-5, OPA-6, OPA-7, OPA-8, OPA-9and OPA-10) were used in the amplification of DNA from the six species of earthworms (*Eisenia fetida, Metaphire posthuma, Eutypheous incommodus, Lampito mauritii, Perionyx simlaensis* and *Dichogaster bolaui*). Based on amplified primers it is concluded that OPA-6 primer, revealed slight differences in *Eisenia fetida* from other species like *P. simlaensis* and *D. bolaui*. Primer OPA-8 revealed a monomorphic pattern of bands starting from 250bp to 750bp in five species (*E. fetida, M. posthuman, E. incommodus, L. mauritti, P. simalaensis*).

***Key words*:** Distribution, Genetic diversity, Earthworms, Vermicomposting, Local species, Eastern Haryana

Earthworms are one of the most important and beneficial macro fauna used extensively in organic farming. Presently 426 species and subspecies have been reported from. Indian mainland which constitutes about 10.6% of total earthworm species known globally [1-2]. According to Julka *et al*. [3] about 89% of the species are endemic to the country whereas the remaining are exotic. The last exploration of earthworm fauna in the state Haryana is done by [4-5]. A total 12 species were identified from various pedoecosystems. Earthworms along with other microorganisms dramatically alter soil structure, water movement, nutrient dynamics and plant growth, in terms of biomass and overall activity [6-8]. The main important role of this eco-friendly species is in vermicomposting (production of compost or organic manure by the culturing of earthworms). Various exotic and endemic species of earthworms are extensively used in various parts of India for vermicomposting including Haryana [9-11]. For vermiculture the preference of species is mainly characterized by short life cycle, high reproduction rate, and high adaptability to organic wastes [12-13]. Not all earthworm species are used for vermicomposting. Most vermicomposting experiments have used epigeic earthworm species because they possess higher composting efficiency. The aim of this study is to culture local species of earthworm in order to evaluate the vermicomposting potential of local species under tropical conditions. The genetic make-up of the species and environmental factors has profound influence on the efficiency of the bioconversion process. Molecular methods become powerful and precise tools for identification and analysis of genetic diversity of earthworm species. The PCR method is known to discriminate between closely related species. For assessing genetic diversity molecular markers such as Random Amplified Polymorphic DNA (RAPD), Restriction Fragment

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Length Polymorphism (RFLP), and Simple Sequence (SSR) are commonly utilized [14]. Molecular markers based on the DNA sequence are more varied and reliable. The PCR-based molecular marker approach requires very less DNA, and is technically simple and cheaper. Molecular markers are considered to provide the best estimates of genetic diversity. But due to the structural simplicity of these invertebrates, earthworm taxonomy is somewhat limited. Moreover, there are scarce fossil records [15] and therefore, it has been difficult to describe ancestral and evolved characters. Few molecular analysis tools relatively have been developed for earthworms, despite the economic and ecological importance of these soil ecosystem engineers [16]. Sharma *et al*. [17] studied molecular characterizations of 24 earthworm individuals collected from diverse locations to assess the level of genetic variation. Meenatchi *et al*. [18] studied the genetic variation among different strains of *Eugeniae eudrilus*.

Presently no information is available on the genetic variation of various species of earthworms from Haryana state (India), till date, so we tried to carry out this experiment on genetic variations in different earthworms. To better understand the genetic variation at the species level, methods are needed that can detect rapidly-changing regions of DNA and to enable population genetic studies. Therefore, the present study attempts to use the RAPD- PCR technique to amplify genomic DNA by using a random primer to quantify the extent of genetic variation in strains collected from different parts of eastern Haryana (India). Other aim of this paper is to update the existing knowledge on earthworm communities and culturing of local species of earthworm of eastern Haryana, a main agricultural area of India facing a problem of fast deteriorating lands due to use of chemical fertilizers.

MATERIALS AND METHODS

Study area: Present study was conducted in Eastern part of Haryana state (Kurukshetra and Yamunanagar district,) (Fig 1) Haryana is situated between 29.0588° North and 76.0856° East. The total geographical area of the state is about 44,212 km^2 which constitute about 1.34% of the geographical area of the country. The climate of Haryana is arid to semiarid with average rainfall of 354.5mm. Both district of eastern Haryana Kurukshetra lies 260 m and Yamunanagar 255m above sea.

Earthworm sampling

Identification

Earthworms were collected from the selected sites by digging method. Collected worms then washed in water and then preserved in 5% formalin. The preserved specimens were identified by following monographs Gates [19] and Julka [20]. The samples were got confirmed by Dr. R. Paliwal, Scientist at Zoological Survey of India, Solan, Himachal Pradesh.

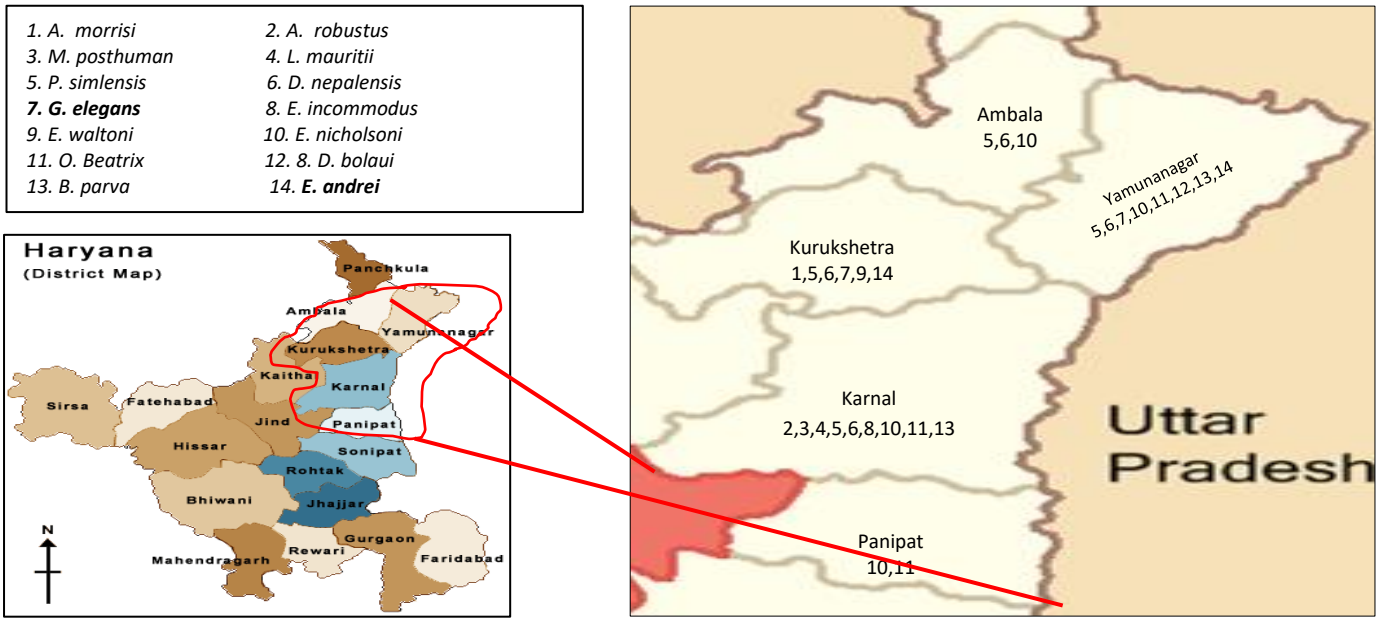


Fig 1 Distribution of earthworm species in districts of Eastern Haryana

Culturing: Earthworms were collected-from different geographical locations of Haryana (Table 1) and brought to the laboratory at Kurukshetra University, Kurukshetra (Haryana) (29º6' N, 76º5' E). For genetic diversity experiment earthworms were cultured in circular plastic containers (28cm diameter and 30 cm depth) kept inside vermiculture house. The below mentioned earthworm species were maintained in the culture room by providing requisite feed substrates for continuous availability for experimentation. Epigeic earthworms were maintained in mixture of decaying organic matter with cow dung and endogeic and anecic earthworms were reared on mineral soil and organic matter mixture. During diversity survey the species found in abundance was chosen for vermiculture. The organic waste material was collected from hostel mess of Kurukshetra University, Kurukshetra, Haryana. The kitchen waste as a feed used for inoculation of earthworm for the production of vermicompost. The C/N and C/P ratio was measured at initial, after 15day intervals. Nutrient estimations involved Walkley and Black [21] method for organic carbon method and Macro-kjeldahl method for total nitrogen. The pH of the bedding material was recorded 6.5- 8.5. The moisture content was 40-60%. Culture beds were covered with jute bags to prevent predators. Percent nutrient changes were calculated by using following formula:

$$[(A - B / A) \times 100]$$

Where A= value in the worm-worked substrate and
B= value in the control substrate
Data were subjected to paired student t- test
The level of significance was set at 0.05.

Table 1 Earthworm species used in the study

Group	Species and location
Group 1	*Eisenia fetida* Kurukshetra cattle shed
Group 2	*Eisenia fetida* Karnal
Group 3	*Metaphira posthuma* Karnal sewage
Group 4	*Metaphira posthuma* YamunaNagar rice field
Group 5	*Eutyphoeus incommudus* Karnal Garden
Group 6	*Eutyphoeus incommudus* Panipat sewage
Group 7	*Lampito mauritti* YamunaNagar non-cultivated area
Group 8	*Lampito mauritti* Karnal tree plantation
Group 9	*Perionyx simalaensis* Yamunanagar irrigation channel
Group 10	*Dichogaster bolaui* Kurukshetra sewage

Isolation of DNA for genetic diversity study

DNA isolation was carried out by using the CTAB method [22] with some slight modifications. The earthworm samples were kept in water to remove soil before proceeding to DNA extraction. The earthworms were anaesthetized by soaking in 70% ethanol, and then washed with distilled water to remove soil debris. All samples were stored in 70% ethanol until DNA extraction. The tissue samples were lyophilized and ground to fine powder using liquid nitrogen in lysis buffer (1% CTAB [hexadecyltrimethyl ammonium bromide], 5% polyvinyl pyrrolidone, 1.4 M Nacl, 20mM-2 mercaptoethanol). The samples were treated with proteinase K (10mg/µl) and incubated in dry bath at 65 °C overnight. The tissue sample was transferred into the eppendorfs and treated with equal volume (800 µl) of Phenol: Chloroform: Isoamylalcohol (P:C:I) and mixed well by inversion. The contents were centrifuged twice at 10000 rpm for 15 min. After centrifugation the supernatant obtained was transferred to another eppendorf and 2/3rd volumes of ice-cold isopropanol was added and mixed well by inverting the eppendorfs in order to precipitate the nucleic acids. RNase A (10mg/µl) was added to prevent contamination by RNA and again incubated overnight at -20 °C. After incubation, the eppendorfs containing the contents were centrifuged at 10000 rpm for 10 min. The supernatant was discarded and the pellet containing DNA was retained and rinsed repeatedly with 500 µl of 70% ethanol. DNA pellet was resuspended in 50 µl of TE (10 mM tris HCl (pH 8.0) + 1mM EDTA) buffer and stored at -20 °C until further use.

The concentration of DNA was assessed by nanodrop and integrity was checked by gel electrophoresis on 0.8% agarose gel. To test the integrity and quantity of DNA, samples were run on 0.8% agarose gel in 1X TAE buffer and stained with ethidium bromide. Good quality and adequate amount of DNA (OD 260/280=1.6 to 1.8) was obtained after treating the tissue sample with a detergent CTAB, Proteinase K and RNase A for prolonged duration of time.

Table 2 Arbitrary primers used for genetic diversity assessment among earthworm strains from different geographical locations of Haryana (India)

Primer	Sequence (5' to 3')
OPA 1	CAGGCCCTTC
OPA 2	TGCCGAGCTG
OPA 3	AGTCAGCCAC
OPA 4	AATCGGGCTG
OPA 5	AGGGGTCTTG
OPA 6	GGTCCCTGAC
OPA 7	GAACGGGTTG
OPA 8	GTGACGTAGG

PCR amplification

The polymerase chain reaction (PCR) is a molecular technology used to amplify a single or a few copies of a piece of DNA across numerous orders of magnitude, producing thousands of copies of a specific DNA sequence [23]. In the present investigation, Random Amplified Polymorphic DNA (RAPD) and molecular phylogeny of earthworms by amplifying genomic 18s rRNA region were used as molecular markers for DNA diversity analysis of earthworm individuals. 10 primers were selected on the basis of reproducibility and used for genotyping of all the earthworm isolates (Table 2).

PCR amplification was performed using Biorad thermal cycler. PCR reactions were set up in a final volume of 25 µl having 10 X PCR mixtures and 10pmol/µl each of primer. PCR cycling conditions for different primers were 94 °C for 3 min, followed by a cycling programme of 35 cycles for DNA denaturation at 94 °C for 1 min, primer annealing at 37 °C for 1 min and extension at 72 °C for 2 min .The reactions were ended with a final extension at 72 °C for 10 min. PCR negative controls were set for all the PCR reactions to negate the chances of contamination in end products and for ascertaining the authenticity of PCR amplifications. PCR products were visualized on 0.8% agarose gel in 1x TAE buffer containing 0.5µg/ml of ethidium bromide and migrated at 100V for 20min. 25 µl of PCR reaction was prepared by adding following components in this order (Table 3).

Table 3 Conditions for amplification

Components	Volume (µl)
10x PCR mixture	12.5
DNA template	1
Primer	1
Nuclease free water	10.5
Total	25

RESULTS AND DISCUSSION

Diversity

During random survey in different pedoecosystem of eastern Haryana, two new species were recorded from Kurukshetra and Yamunanagar districts. This was the first record from this studied area. Both species were recorded very less in number. Apart from this a total of 12 species were recorded previously from eastern Haryana districts (Fig 1).

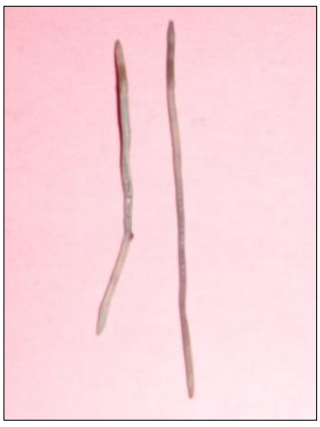


Fig 2 *Gordiodrilus elegan* [24] Beddard 1892

Fig 3 *Eisenia andrei* [25] (Bouche 1972)

Gordiodrilus elegan, Beddard 1892 [24]

They are belonging to family Ocnerodrillidae. It is an introduced species and probably originated from tropical regions

of Africa [19]. From Kurukshetra and Yamunanagar district of Haryana the species was recorded from sewage pedoecosystem at the depth 5- 10 cm where litter is mixed with soil. Body is light pigmented. Body size ranges from 26-55mm×0.8-1.7mm (Fig 2).

Eisenia andrei Bouche 1972 [25]

This species of earthworm belongs to family Lumbricidae. Mainly feed on a dead organic matter. Body is red purple with brown bands. The body size varies from 60-70 x 3-4mm (Fig 3). It is an epigeic species known for its vermicomposting potential because it is peregrine and ubiquitous with worldwide distribution with temperature tolerant capacity.

Genetic diversity

The integrity of isolated nucleic acid (DNA) on 0.8% agarose gel is shown in a (Fig 1). The content of high molecular weight (non-degraded) DNA was higher. Thus, the double extraction seemed more appropriate than the single extraction for DNA amplification. There was no evidence of RNA in these samples. However, partly degraded DNA was obvious as faint fast-moving bands slightly below the mid part. Thus, the phenol-chloroform extraction, and particularly the double extraction using CTAB method, successfully extracted a large quantity of high molecular weight DNA without heavy contamination of degraded DNA (Fig 4).

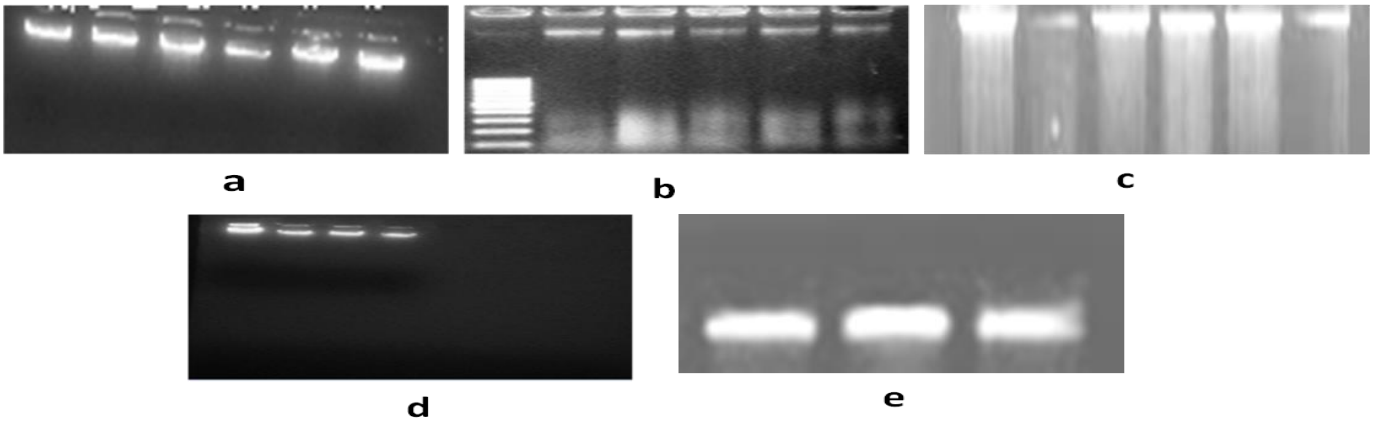


Fig 4 Integrity of the isolated DNA samples on 0.8% agarose gel

Results of amplification (Random amplified polymorphic DNA)

RAPD is very sensitive to the reaction conditions and the reproducibility has to be improved by standardising every step of the analysis [26]. We have repeated the amplification with the same template DNA 2–3 times and we used only those results that showed consistent patterns among repeated amplifications of the same individual. The present findings suggest that species-specific RAPD characters may also exist for the six species of earthworm *Eisenia fetida*, *Metaphira posthuma*, *Eutypheous incommodus*, *Lampito mauritti*, *Perionyx simlaensis* and *Dichogaster bolaui*. Ten-base primers were used in the amplification of DNA from the six species of earthworm (OPA-1, OPA-2, OPA-3, OPA-4, OPA-5, OPA-6, OPA-7, OPA-8, OPA-9 and OPA-10). Amplification of primer OPA-1 revealed no amplification in lane 1, 2, 5 and 10 (*E. fetida*, *E. incommodus* and *D. bolaui*) but most of the bands were common in other species with one additional band in lane 6 (Figure 5). Amplification of primer OPA-2 revealed the common pattern for most of the bands in all the six species obtained from different geographic locations (Fig 6).

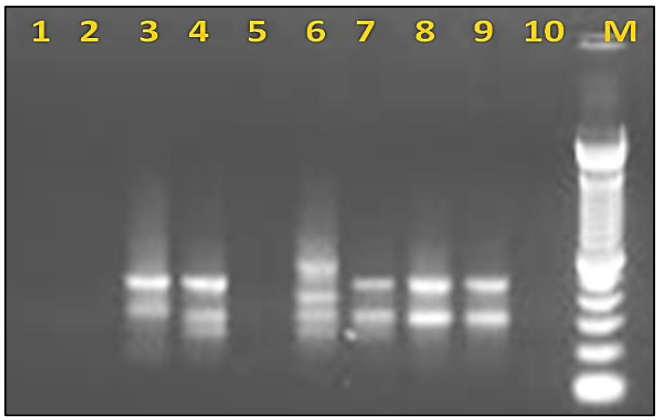


Fig 5 Amplification products of primer OPA-1. Lane 1-Group1; Lane 2-Group2; Lane 3-Group3; Lane 4-Group4; Lane 5-Group5; Lane 6-Group6; Lane 7-Group7; Lane 8-Group8; Lane 9-Group9; M indicates Molecular marker

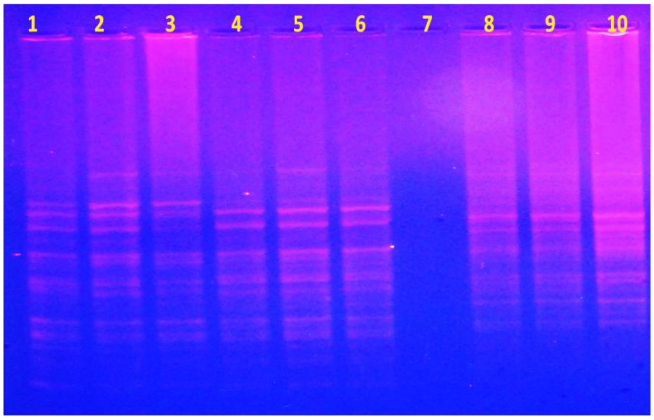


Fig 6 Amplification products of primer OPA-2. Lane 1-Group1; Lane 2-Group2; Lane 3-Group3; Lane 4-Group4; Lane 5-Group5; Lane 6-Group6; Lane 7-Group7; Lane 8-Group8; Lane 9-Group9; Lane 10-Group10

Only one, OPA-6, revealed slight differences between all these species (Fig 7). In this primer, the amplification products of *Eisenia fetida* were slightly different from other species like *Perionyx simlaensis* and *Dichogaster bolaui*. Not many differences between the same species of different locations were observed but significant differences between the location and the number of bands between the six species were observed. As we used only few individuals of each species, these results should be considered as very preliminary. This primer may be a useful marker of specific populations of this species, although further research into this field needs to be carried out.

Primer OPA-8 revealed monomorphic pattern of bands starting from 250bp to 750bp in five species (*Eisenia fetida*; *Metaphire posthuma*; *E. incommudus*; *Lampito mauritti*; *Prothemenops*). A minor difference in the band intensity was observed between these species. There were two major bands of approximately 550bp and 750bp in all the species and other bands were dim in intensity (Fig 8).

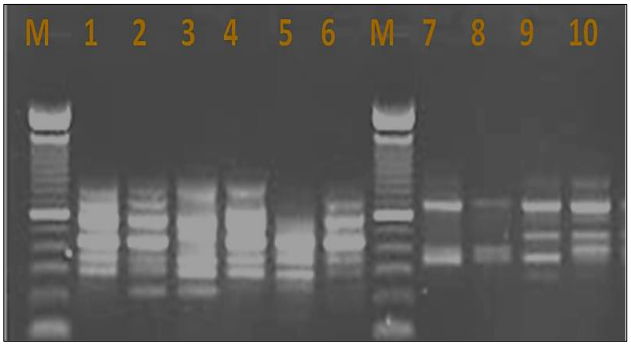


Fig 7 Amplification products of primer OPA-6. Lane 1-Group1; Lane 2-Group2; Lane 3-Group3; Lane 4-Group4; Lane 5-Group5; Lane 6-Group6; Lane 7-Group7; Lane 8-Group8; Lane 9-Group9; Lane 10-Group10. M indicates molecular marker

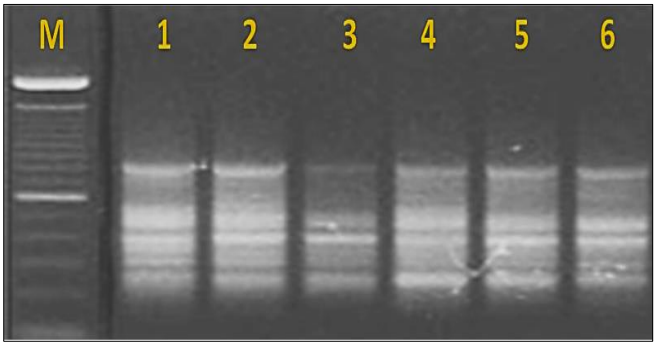


Fig 8 Amplification products of primer OPA-8. M- Molecular marker; Lane 1- *Eisenia fetida*; Lane 2- *Metaphire posthuma*; Lane 3 – *E. incommodus*; Lane 4- *Lampito mauritti*; Lane 5 – *Prothemenops simalaensis*; Lane 6- *Dichogaster bolaui*

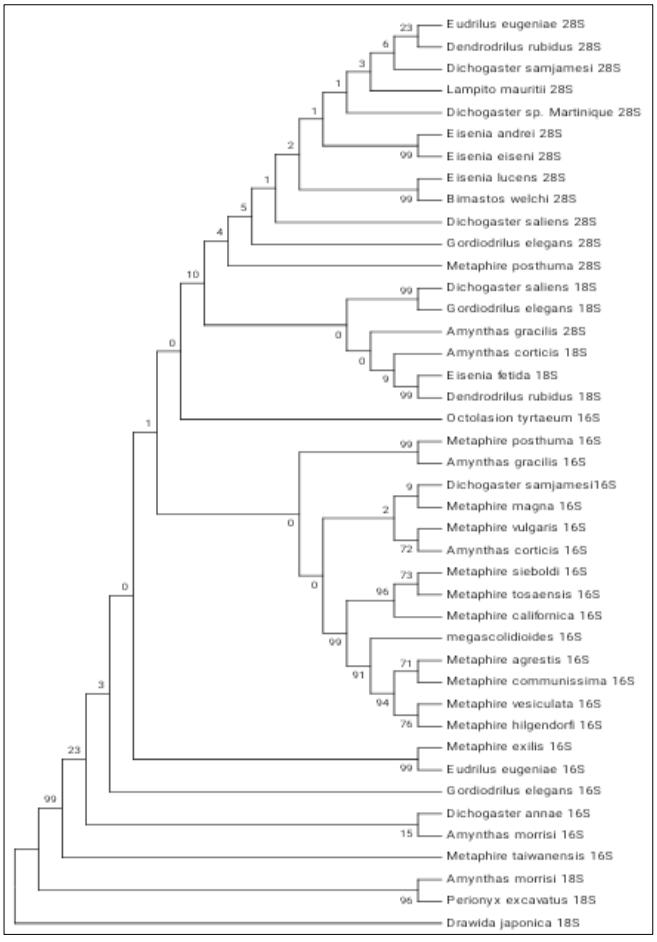


Fig 9 Phylogenetic relationships of 16S, 18S and 28S rRNA between different strains of earthworm

Phylogenetic analysis

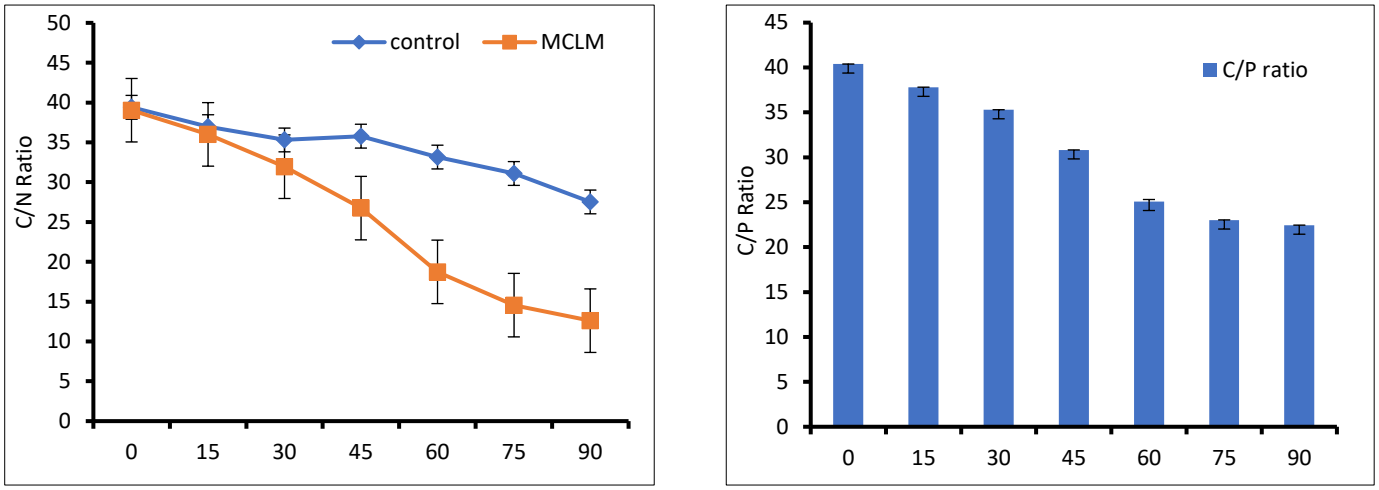
For phylogenetic analysis, available sequences of different earthworm species were obtained from the NCBI protein database. The evolutionary history was inferred using the Maximum Parsimony method. The boot strap consensus tree inferred from many replicates is taken to represent the evolutionary history of the taxa analyzed [27]. Branches corresponding to partitions reproduced in less than 50% boot strap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap are shown next to the branches [27]. Evolutionary analyses were conducted in MEGA6 [28]. Phylogenetic tree was constructed by the software showing the ancestral relationship among the sequences (Fig 9).

Vermiculture

Our studies aimed at a local species found from various pedo-ecosystems studied in Haryana. During random survey period *L. mauritii* was the only worm found throughout the year from almost all studied sites (Cultivated, non-cultivated, garden, grassland and sewage). Of course, it was purely anecic worm which show efficiency for vermiculture. Hence in our experiment the preference was given to this indigenous species for vermicomposting. Monoculture of *Lampito mauritii* in kitchen waste for 90 days showed significant changes in nutrient profile. The individual mass (mg) on the start of experiment was 466 ± 4.04mg, and on the end of experiment the mass was recorded 798.6 ± 4.25mg. Total cocoon production was found to be 29.9 ± 1.52. Reproduction rate of this worm (cocoon/worm/weak) was recorded 1.39 ± 0.54. After 90 days of inoculation a fully decomposed and matured vermicompost which is brown blackish in colour and its moisture content is 18-25% was prepared. Fig (10 A-B) depicts the decrease in C/N and C/P ratio of the kitchen waste vermibed.

During random survey two species were recorded for the first time from Kurukshetra and Yamunanagar district of Haryana. First is *Gordiodrilus elegan* showed the habitat preference as it was collected only from sewage region of this studied area of Haryana. The precise record of less than 10 specimens was found from sewage site. The main home of this species is tropical Africa. But in India it was found from south India, Punjab and some other parts. From Rajasthan it was recorded from Domestic sewage and orchards [29-30]. Second one is *Eisenia andrei* do not found in natural habitat like other species recorded from this trans-Gangetic region of Haryana, only in heap of animal dung. The distribution of particular species and its absence from other habitats depicts the diversity. Their diversity was comparatively poor as compared to other earthworm species found from this region indicating that life supporting for dispersal and survival is not much available. With this the reported earthworm species was 14 from different pedoecosystem of eastern Haryana [4-5]. The earthworm found

in soils having physico-chemical properties moisture from 22.2-33.80 %, pH 6.5-7.8, temperature 25-30 °C, and organic matter 2.23 - 3.39%, C/N ratio 8.0 - 9.38 ratio and C/P 29.08. Both species found was epigeic in nature.



A- Composting days for *L. mauritii*

B- Composting days for *L. mauritii*

Fig 10 (A-B) depicts the decrease in C/N and C/P ratio of Kitchen waste by *L. mauritii*

In the present study, analysis of genetic diversity was performed using RAPD molecular marker and evolutionary history based on RNA systems to define genetic relationships among six species of earthworm isolates. The 16S, 18S and 28SrRNA sequence of different earthworm strains was matched with previously reported sequences available at NCBI and a phylogenetic tree was constructed (Fig 7).

The present study indicated that different earthworm strains across the locations spread in Haryana exhibited considerable similarity. Although, a fair amount of genetic variations existed between the six individual species used in this study but the differences were non-significant between the same species spread across different geographical locations. This may be due to the approximately similar climatic conditions, which could have strong bearing on their evolution. Our results are in accordance with the results obtained by Kautenburger [16] who studied genetic structure among earthworms from different sampling sites in Western Germany. In his study he used 40 oligonucleotide RAPD primers, of which three produced high polymorphic banding patterns. He concluded that genetic similarities within and among *Lumbricus terrestris* populations were similar at widely separate locations. Furthermore, our results stand in agreement with Dyer *et al*. [31] who examined four different earthworm species in Australia at different locations with RAPD-PCR. They found that the species *Aporrectodea trapezoides* was genetically very similar, due to parthenogenetic reproduction. Sankar *et al*. [32] showed that *L. mauritti* and *P. excavatus* showed significant differentiation among population on RAPD-PCR analysis which is agreement with our results.

Molecular diagnostic analysis of earthworms is usually performed by sequencing of sub units of rRNA. In present investigation, sequence analysis of different rRNAs of selected individuals belonging to different strains revealed a significant match amongst themselves, which further confirms their identity. Therefore, the present research work showed species-specific groupings of earthworm individuals with high levels of statistical support (bootstrap values). However, although variation was seen within populations collected from specific geographical regions, statistically non-significant data were obtained. Our results show that earthworm strains possess a relatively similar genetic structure within the sampling sites. The results of this study indicate that RAPDs and rRNA sequencing offer a reliable and effective means of assessing genetic variation in earthworms. This is in agreement with Sharma *et al*. [17] who suggested that molecular markers allowed rapid assessment of diversity analysis among closely related isolates of earthworms. This is the pioneer work in eastern part of Haryana and there is a need to carry further studies on this field to draw phylogenetic relatedness among individual of different species so that these species can be used for vermitechnology for sustainable development.

The present study indicated that *Eisenia fetida*, *Metaphira posthuma*, *Eutypheous incommodus*, *Lampito mauritti*, *Perionyx simlaensis* and *Dichogaster bolaui* strains across diverse locations of Haryana, India exhibited considerable amount of genetic variations. This may be due to differential niche requirement and climatic environ, which may be the reason for their evolutionary differences and hence might creating variation within the strains of the earthworms. This study based on molecular characterization (RAPD and 16S, 18S, 28S rRNA analysis) of earthworms is the first report from Haryana, India. Further, various earthworm species could be demonstrated with the help of different markers, which may provide significant information about their genetic variation among earthworms, which could be applied for their identification, characterization and conservation of vermiresources in various ecological habitats.

The Kitchen waste substrates subjected to monoculture of *L. mauritii* for 90 days showed notable changes in nutrients. There was decrease in pH of vermibed with the passage time. It may be due to mineralization of N and P [33]. During vermicomposting, the organic carbon in all vermibeds declined. At the end of the experiment the loss of organic carbon (compared to its initial level) was observed for kitchen waste by monoculture of *L. mauritii* (33%). Organic carbon exhibited significant differences over 90 days of vermicomposting as compared to initial day ($P< 0.001$) in the vermibeds. Our data are in agreement to the report of Elvira *et al.* [34] and Sonowal *et al.* [35] who observed loss of organic carbon as CO_2 during vermicomposting of different organic wastes. The combined process (feeding of earthworms on organic matter and microbial

degradation) brought about C loss from substrates and accelerated waste stabilization process. The total N content increased in the vermibed over 90 days of composting. The total N content was found to be 50.4%, when compared to its intial value. The nitrogen content was statistically significant ($P<0.05$). In the processing of organic waste through composting, earthworms accelerated the nitrogen mineralization and subsequently N profile was higher in the end product. The final content of nitrogen in vermicomposting is dependent on initial nitrogen present in the waste and the extent of decomposition [36]. In the present study it could be seen that available P and K also increased but very slowly. The variation in the end product might be associated with the differences in working ecological functioning of anecic worms used in monoculture vermibed. The C/N ratio of vermibed decreased significantly ($P<0.05$) over 90 days of vermicomposting. The C/N ratio of the vermicompost is lowers indicating most suitable for plant growth [37]. The anecic (*L. mauritii*) species is capable of both organic waste consumption as well as of modifying the soil structure, In the present study, when species were cultured in kitchen waste gave better results in terms of decomposition and mineralization as compared to control.

CONCLUSION

It is concluded that the earthworm data increased from previous data by giving new record of two species (*G. elegan* and *E. andrei*). Based on genetic study it was concluded that there was a relative similar genetic structure of earthworms within sampling sites. This species belongs to different ecological categories but still can survive in similar climatic condition of Haryana. A local species can replace other epigeic earthworms for vermiculture for the production of organic manure which in turn help in sustainable development.

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