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Review Article

A REVIEW ON MOLECULAR MARKERS AS TOOLS TO STUDY EARTHWORM DIVERSITY

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ABSTRACT

'Earthworms', the natural creature and unsung heroes of soil have gained much of the scientific attention throughout the world including India to maintain fertility of soil and dietary protein source for domestic animals. Earthworms play an important role in soil ecology and are well recognized as engineers of soil. They occupy a vital position in soil food-webs. Aristotle called them intestines of the earth and considered as important biological resources that have tremendous potential in agricultural ecosystems as they remarkably influences physical structure as well organic matter composition of the soil. Of globally estimated 6500 species of earthworms, only 3500 species are discovered and described. Indian subcontinent has got a massive fauna of Oligochaete, (earthworms) which are represented by 505 species and 67 genera. Molecular markers such as Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), and Simple Sequence (SSR) have proved to be worthy and important in assessing genetic diversity.

Recently DNA barcoding has exposed unexpected number of species that were often impossible to be isolated on morphological basis. The present study reviewed the diversity of earthworms with integrated approach of taxonomy including molecular markers.

Keywords: Earthworms; Molecular diversity; Molecular markers; DNA barcoding, Threat to diversity of earthworms; Endangered species of earthworms

INTRODUCTION

The subject may appear an insignificant one, but we shall see that it possesses some interest; and the maximum 'de minimis lex non curat' (the law is not concerned with trifles) does not apply to science.

Earthworms engage a unique position in animal kingdom and are the first group of multi-cellular and eucoelomate invertebrates who have succeeded to occupy terrestrial environment. The knowledge about the importance of earthworms is not a very modern phenomenon. The Greek philosopher Aristotle (384 -322 B.C.) regarded worms as the intestines of the earth. These intestines of the earth are regarded as vital biological resources that have tremendous potential in agricultural ecosystems as they notably influence the physical structure as well as the organic matter composition and hence endorse the growth of plants (Lee, 1985). Cleopatra (69-30 B.C.) recognized the earthworm's role to Egyptian agriculture and declared earthworms to be sanctified. In Egypt, the punishment for the removal of earthworms was death. Egyptian farmers were not even allowed to touch an earthworm with the fear of offending the god of fertility. The credit for the excessive fertility of the Nile valley largely goes to earthworms. Knowledge regarding the eco-friendly role of earthworms in pedogenesis and soil fertility did not emerge until the late eighteenth century. Charles Darwin (1809-1882) studied earthworms for more than forty years and dedicated an entire book "The Formation of Vegetable Mould through the Action of Worms". Darwin quoted, "It may be doubted that, there are any other animals which have played so important part in the history of the world as have these lowly organized creatures".

Earthworms have the potential to biodegrade and bio-transform' chemical pollutants, there by converting them to less toxic substances in their bodies. Earthworms play an important role in soil ecology as they have the tendency to reach high densities. They are recognized as soil engineers, and occupy a vital position in soil food-webs. They improve the structure of the soil by tilting and mixing, play important role in humus formation. Earthworms also play important role in increasing the water holding capacity of the soil.

A large number of research papers have been published regarding the diversity studies of earthworms using molecular markers where, the emphasis was given on the individual markers. So keeping these things in view present

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study has sum up all the molecular techniques used for studying the earthworm and other oligochaete diversity. That may be helpful for the researchers working in the field of molecular diversity and assessing the molecular diversity of earthworms with use of integrated molecular tools. There may be an affluent source of variation occurring at the molecular level including population genetics, evolutionary biology and molecular ecology.

On the basis of morpho-ecological habitat the species of earthworms are as epigiec, endogeic and anecics (Bouche, 1977). Epigeic species are the ones that inhabit the top soil rich in decaying organic matter. The rate of feeding on the undecomposed litter is higher in these groups of earthworms producing high fertile soil due to the conversion of large amount of organic litter.

Endogeic species inhabit the subsurface and prefer to feed on soil that is rich in organic matter and usually form burrows that are horizontal. Aanecics builds burrows that are permanent and vertical and deeply penetrate the soil. These worms play a vital role in litter decomposition, pedogenesis, cycling of the nutrients and entail less confrontation for the growth of roots.

In present days earthworms have gained more scientific attention throughout the world including India the reason being their use in vermicomposting and also as a dietary protein source for domestic animals. Though, merely half a dozen of known 505 species of earthworms in India are regularly used for vermiculture and compositing (Julka and Paliwal, 2005).

Earthworms are considered an important soil macro fauna, and have intense effects on ecosystems. Due to the beneficial effects of these soil macro fauna they have fascinated a lot of attention, particularly in the agricultural sectors.

Besides constituting more than 80% of the total soil biomass, only a half of the known earthworm species have been identified so far. This publication will provide all these techniques that will be used by the researchers throughout the globe to identify these natural bioreactors. In this article the authors have tried to provide a framework of the techniques that will prove beneficial to identify a large pool of unidentified species of earthworms, and will help the researchers throughout the world to identify the remaining ones.

EARTHWORM DIVERSITY

Global scenario

The distribution and profusion of earthworms rely on a number of factors such as soil and climatic parameters and crop management practices. Density and biomass of the earthworms are directly related to the annual rainfall and inversely correlated to the texture of soil i.e., greater the rainfall greater will be the density and biomass of the earth worms and more the sand content of the soil less will be the density and biomass (Buckerfield *et al.,* 1997).

Conventional management practices have led to reduced density and diversity of earthworms the reason being earthworms are sensitive to minute climate changes and food availability. Moreover (Paoletti 1999) and (Curry *et al.,* 2002) recorded lesser population in cultivated soils than in uninterrupted habitats or forest ecosystems. According to (Fragoso *et al.,* 1997) out of 6500 estimated species of earthworms, only 3500 species are discovered and described worldwide.

(Munnoli *et al.,* 2010) have reassessed the work of a number of researchers, that have reported an ample earthworm diversity throughout the world biodiversity, for example in Japan (over 70 species), New Zealand (192 species), Libya (3 species), Australia (300 species), Pakistan (15 species), British Isles (44 species), France (180 species), Canada (20 species), and India (505 species).

Earthworm size varies greatly, in India; some species are less than 20 mm e.g. *Bimastos parvus* (Eisen), *Microscolex phosphoreus* (Drugs), *Dichogaster saliens* (Beddard), while others species, *Drawida nilamburensis* (Bourne) may reach up to 1 m in length. *Megascoledis australis* (McCoy) native of Australia is reported to reach a size in excess of 4 m while *Microchaetus microchaetus* (Rapp) attains a length up to 7 m (Mohammad, 1993).

Distribution specificity of earthworm species in diverse pedoecosystems all over the world is determined by their presence or absence. Northern America is considered as the biodiversity hot spot of the earthworms which chiefly belong to two families *Lumbricidae* and *Megascolecidae* (Fender, 1995).

Sanchez-De Leon et al., 2003 studied modifications of earthworm diversity in neglected tropical pastures (Puerto Rico) USA and observed seven species viz., *Neotrigaster rufa* (Megascolecidae), *Amynthas gracilis* (Megascolecidae), *Estherella sp.* (Glossoscolecidae), *Borgesia sedecimsetae* (Megascolecidae), *Trigaster longissimus* (Megascolecidae) and *Pontoscolex corethrurus* (Glossoscolecidae).

The initial study of the diversity of earthworms in the pastures and forests of Colombian Andes was conducted (Martinez et al., 2006). They observed/ discovered 13 genera and 18 earthworm species. Forests with (13) species of earthworms were rich both in number as well as biomass than in pastures with (9) species, however four species were common to both forests and pastures. Till date the tropical ecosystems, the forests were documented to be the richest in species abundance. Some of the earthworm species namely *Maipure agricola, M. savanicola,, Amynthas cortices, A. gracilis, Martiodrilus heterostichon, Dendrobaena octaedra, Pontoscolex corethrurus* are the probable indicators of agricultural soils or alterations in agro ecosystem, owing to their elevated adaptation capacity for more biomass.

Fourteen species of earthworms were reported in Jaguapita region of State Parana, Brazil (Nunes et al., 2006). Of which 4 species viz., *Glossoscolex n. sp., Fimoscolex n. sp.,* (Glossoscolecidae), *Ocnerodrilide n. sp., Belladrilus n. sp.* 1(Ocnerodrilide) are native, two Ocnerodrilid species (*Eukerria* spp.) were unidentified and the remaining 8 *Eukeria eiseniana, E. saltensis, Dichogaster affinis, D. bolaui, D. Saliens (Acanthodrilidae), Pontoscolex corethrurus (Glossoscolecidae), Amynthas sp. (Megascolecidae), Ocerodrilus occidentalis (Ocnerodrilidae*) were exotic. In the exhaustively managed systems Aporrectodea species were the most frequent however the other species were common in the less exhaustively managed ecosystems. (Ghafoor *et al*., 2008) reported the earthworm biodiversity from Pakistan. Grass lands, river bank, forest ecosystems and cultivated land were the areas used to study the burrowing action and the virtual profusion of the earthworms. The species recorded from these areas characterized five families, 12 genera and 20 species. The percentage compositions of these families were *Moniligastridae* 8%, *Lumbricidae* 12.05%, *Megascolecidae* 73.30%, *Tubificidae* 3.23% and *Naididae* 3.35%. *Pheretima posthuma, P. hawayana*, *P. Morrisi* were the three species of Megascolecidae that were active and mainly abundant species in diverse habitats during early moon soon months (July-August).

The diversity and distribution of the global earthworms in Thailand were studied by (Somniyam and Suwanwaree, 2009) and a total of 19 species belonging to *Glossoscolecidae*, *Ocnerodrilidae*, *Moniligastridae*, Octochaetidae and Megascolecidae families were found. The species found were Amynthas alexandri, *A. tokioensis*, *A. cortices*, *Metaphire bahli*, *M. peguana*, *M. posthuma*, *M. Planate*, *M. houlleti*, *Pontoscolex corethrurus*, *P. elongate*, *Dichogaster affinis*, *Di. Bolaui*, *Di. modiglianii*, *Drawida sp.*, *D. beddardi*, and *Gordiodrilus elegans.*

Nurhidayati et al., 2011 carried out the studies related to the consequence of soil management both in the presence and absence of organic matter inputs on the earthworm density and diversity, relating the characteristic features of the soil in sugarcane growing areas of Indonesia by different annual rainfall patterns. *Pontoscolex corethrurus* (Glossoscolecidae) and *Pheretima minima* (Megascolecidae) were the species abundantly recorded in the sugarcane fields. The evaluation of earthworms namely biomass, density, number, average weight, and diversity index were affected by the variation in yearly rain fall and lacking organic matter contributions/ inputs possess inferior appraisals. The density and biomass of earthworms in the sugarcane areas were increased mainly by the organic matter inputs however the density and the biomass was still lower than that of the forest area.

Other chemical properties of the soil such as total nitrogen and the organic carbon also influenced the density and biomass of earthworm, a 25% increase in total soil nitrogen documented an increase in earthworm density and biomass by 79% and 75% respectively, however an increase in soil organic carbon by 25% resulted 64% and 83% increase in the earthworm density and biomass respectively (Nurhidayati *et al.,* 2011).

Indian Scenario

A number of the biologists all over the world are studying the earthworm biodiversity (Tsai *et al.,* 2000; Blakemore, 2003; Blakemore *et al.,* 2006; Sautter *et al.,* 2006). In India Western Ghats and Eastern Himalaya regions are recognized as biodiversity hot spots mainly because of their species richness. Though the area cover only 2% of the world's landmass, these regions shore up about 10.5% of the entire known worldwide earthworm diversity (Julka, 2010). West coast regions and Western Ghats are prosperous in the earthworm diversity with 219 recognized species which contribute about 43.366% of the overall earthworm species in India. India has a very high percentage of endemic population, together at genus and species level; about 71% of genera and 89% of earthworm species are native. Besides the endemic, a number of exotic ambulant species of earthworms are also found which are currently widespread in distressed habitats due to subsequent deforestation and rigorous cultivation (Julka, 2008). Reproductive strategies of earthworm population are the main indicators that help to determine the genetic diversity of these creatures. Consequently, in parthenogenetic species small clonal variability can occur if a single or few creator worms (cocoons) have arrived and established a population at a location. In *amphimictic* species (capable of interbreeding freely and producing fertile off springs), inbreeding can also change the genetic diversity in a population. The diagnostics of earthworms conventionally depend on morphological traits viz., colour, shape, number of body segments, position of clitellum, etc. The classification, handling and grouping of number of earthworm species is over and over again a difficult/ponderous job.

According to (Julka, 1993) the subcontinent of India has got a massive fauna of *Oligochaete*, (earthworms) which are represented by 509 species and 67 genera. (Blanchart and Julka 1997) considered the effect of the human distributions and anthropogenic activities (human impact) on the communities of earthworms collected at the end of monsoon seasons from a wide array of environments. They recorded 28 species of earthworms belonging to the three different families of *Megascolecidae*, *Moniligastridae*, *Octochaetidae*, with each family containing three (3), seven (7) and eighteen (18) species respectively. There was no clear association between the community vegetations and the characteristics, whether the forests and pastures sustain high or low organic matter or soil properties. However, few species were limited to forest ecosystems, some to pastures while others were present in all environments/ ecosystems (Blanchart and Julka 1997).

(Sinha *et al.,* 2003) assessed impact of environment, management of water and its physico-chemical factors and organic input quality on abundance and diversity of earthworms from the Hariyali Devi sacred landscape of Garhwal Himalaya in Uttarakhand State of India. Total seven species viz., *lumbricid Allolobophora parva* (Eisen), *moniligastrid Drawida nepalensis* (Michaelsen), *megascolecid Eutyphoeus sp.* (Eutyphoeus pharpingianus), *octochaetid Octochaetona beatrix* (Beddard), *megascolecid Amynthas corticis* (Baird), *megascolecid Periyonx sp.*, *octochaetid Lennogaster pusillus* (Stephenson) were found in the landscape belonging to four families. D. nepalensis had the cosmopolitan in distribution. *Eutyphoeus sp.* and *A. parva, Periyonx sp.* were restricted to the forest ecosystems and *A. corticis* and *L. Pusillus* only to agricultural ecosystems.

Tripathi and Bhardwaj, 2005 studied the earthworm biodiversity in the dry regions of Jodhpur district (Rajasthan, India). From this deserted ecosystem a total of nine species of earthworms viz., *Amynthas morrisi*, *Ramiella bishambari*, *Ocnerodrilus occidentalis*, *Pontoscolex corethrurus*, *Metaphire posthuma*, *Lampito mauritii*, *Perionyx sansibaricus*, *Octochaetona paliensis*, *Dichogaster bolaui* were traced all belonging to the Glossoscolicidae, Megascolicidae, Ocnerodrilidae and Octochaetidae families. The species *P. sansibaricus*, *O. paliensis* and *P. corethrurus* were for the first time accounted from the arid regions of Rajasthan.

The earthworm diversity present in Jodhpur district of Rajasthan were either of indigenous/native alien or foreign /exotic alien. The exotic species viz., *A. morrisi*, and *M. Posthuma* were extensively distributed to the arid areas. Similarly the native species like viz., *L. mauritii* were also spread to the arid and desert conditions. These species seem to be resistant to drought conditions. Sathianarayanan and Khan, 2006 studied the distribution pattern and population densities of earthworm fauna in the regions of Pondicherry.

A total of ten species of earthworms' viz., *Drawida limella*, *D. scandens*, *D. willsi*, *Pontodrilus bermudensis*, *Octochaetona serrate*, *O. Barnes*, *Pontoscolex corethrurs*, *Perionyx excavatus*, *Lampito mauritii* and *Eudrilus eugeniae* were found. These noted species belonged to six families and seven genera. Fourteen areas of diverse habitats were selected and the diversity of the earthworms from these respective areas was presented. In all these habitats *L. mauritii* was found to be dominant. On the basis of population densities and age group, the density among different species was found to be maximum in vermiculture areas with 358 and lowest 25 in halophilic habitats.

Chaudhari *et al.,* 2008 carried out the investigation of earthworm species, with reference to biomass, density, frequency, diversity, and distribution in the rubber plantation areas of Tripura state of India. These investigations revealed presence of a minimum of 20 species belonging to five families (*Megascolecidae, Moniligastridae, Glossoscolecidae, Octochaetidae, Ocnerodrilidae*). Pontoscolex corethrurus, species was found to be the dominating one, signifying 72% density and 61.5% biomass of the total earthworm population. Suthar, 2009 while conducting the studies related to diversity of earthworms in the northern India reported maximum number of worms from the areas where the farmers have followed integrated farming practices (100%), followed by management through fertilizers (70%) and conventional farming practices (18.9%).

Studies related to the diversity and distribution of the endemic and exotic worms in the natural and regenerating ecosystems of Central Himalayas of India were carried out by (Bhadauria *et al.,* 2000).

Comparative study regarding different communities of earthworms collected from climax forest, sub climax mixed forest, 6 and 40 year-old pine forests and regenerating open grassland locations was done to understand the impact of deprivation and deforestation of natural forest places. Four families of earthworm species viz., *Lumbricidae, Octochaetidae, Megascolecidae* and *Moniligastridae* consisting of eight species (*Bimostus parvus, Octolasion tyrtaeum*), *Octochaetona beatrix*), (*Amynthas cortices, Eutyphoeus festivus, E. nanianus, E. waltonii*) and (*Drawida sp.*) were recorded respectively.

The four decade old pine forest is believed to contain the maximum number of species. A significant correlation was established between the population size and the physio-chemical factors such as temperature, soil moisture and organic matter. (Verma *et al.,* 2010), based on the extensive study carried out in the Gangetic plains of the Uttar Pradesh, during late monsoons (August-October) a total of 11 taxa of earthworms viz., *Metaphire anomala, M. birmanica, M. posthuma, Eutyphoeus orientalis, E. waltoni, E. incommodus, E. pharpingianus, Lampito mauritii, Pellogaster bengalensis, Polypheretima elongate, Perionyx sansibaricus* belonging to 6 genera and 2 families were commonly found. (Shylesh Chandran *et al.,* 2012) carried out the investigation of earthworms in the Nilgiri Biosphere reserve (NBR). The study concluded that 84.67% of the species identified were native and the rest were exotic.

Haokip and Singh, 2012 assessed the diversity and occurrence of different species of earthworms in some selected forest areas. After assessing the diversity and occurrence a comparative study of earthworm communities was done in sub-tropical forest ecosystem in order to recognize the impact of biotic disorder. Three sampling sites were selected: i) a sub-tropical forest ecosystem that is disturbed by the dominance of Oak, ii) reserved forest of natural occurrence and iii) oak plantation area that is dully managed. The results of the study revealed the occurrence of a minimum of 7 earthworm species that belonged to 5 genera and 4 families: *Glossoscolecidae (Pontoscolex corethrurus) and Moniligastridae (Drawida sp.), Megascolecidae (Metaphire houlleti, M. anomala,* *Amynthas corticis, and A. morrisi), Octochaetidae (Eutyphoeus sp.). Glossoscolecidae* and *Megascolecidae* generally occurred in stressed forest ecosystem.

Molecular markers

A tool used for the analysis of genetic diversity: A number of molecular markers such as Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), and Simple Sequence (SSR) are used for fingerprinting (Fang, 1997) which have insightful use in genetic diversity assessment (Agarwal *et al.,* 1999). Advancement in PCR-based markers relies on the use of random short sequence as primers designed for the amplification of random segment of target gene. Amplification of target gene used as molecular markers and identification are based on their presence or absence. The genetic diversity and closeness in any species along with earthworms are assessed by PCR-RAPD techniques that are extremely helpful and potent. The use of RAPD and PCR based markers are beneficial in that it does not require sequence information of a gene to be amplified (Williams *et al.,* 1990). The principal markers such as random amplified polymorphic DNAs (RAPDs; Welsh *et al.,* 1990) and amplified fragment length polymorphisms (AFLPs; Vos *et al*., 1995) are the markers known to contain multiple loci for the reason that they simultaneously generate data from many loci.

Molecular study that intends to advance the understanding related to the earthworm taxonomy has a comparatively short history (Dupont, 2009). To molecular ecologist, the challenging taxonomy of earthworm species is one of the critical drawbacks. In the recent times molecular methods have turn out to be the potent and precise tool that have proved to be very useful to overcome limitations of the conventional visible markers in manuscripting the existing earthworm diversity.

Molecular markers are widely recognized to be ideal tools for the classification and examination of both plant and animal diversity. Of late the implementation of DNA barcoding has exposed unexpected number of species that are often impossible to be separated on phenotypical / morphological basis. The worldwide earthworm DNA barcoding drive based at Canadian centre for DNA barcoding has exposed diversity among several common earthworm pedigrees (James, 2010). Development of a consistent and fast method for the identification of species is vital tool used for studying earthworms.

The identification of earthworms is extremely complex and is only based on diagnostic morphological features (Csuzdi and Zicsi, 2003; Reynolds, 1977; Schwert, 1990).

The latter has also proved to be one of the main problems in taxonomic variations. To overcome this problem cloud molecular method seems to be a possibility. Mitochondrial gene fragments (12s, 16s, COI) are the frequently used for the identification of earthworm species.

Polymerase Chain Reaction technique is known to differentiate among closely connected species and can perceive polymorphism exclusive of any preceding information related to the DNA sequence of that specific organism (Morin *et al.,* 2004). Identification of earthworm species on the basis of morphology is difficult the reason being the lack of the stable and easily scorable diagnostic morphometric characters (Pop *et al.,* 2003) while assessing the genetic diversity in different species of earthworms using molecular methods studied the genetic variations in the exotic earthworm, *Aporectodea sp.* in the soils of Australia by using RAPD technique. The results obtained showed the molecular similarity (M) among individuals of a single population of *Aporectodea trapezoids* was 85.6% and that of intimately connected; bi-parental, *Aporectodea caliginosa* share 77.1% similarity. These species were considered to be closely related on the basis of evidences of morphological classifications.

Dyer *et al.,* 1998 stated that *Eisenia fetida* collected from different sites of Himachal Pradesh, showed greater intra and interspecific population differences by using molecular markers. Group study clearly differentiates earthworms' isolates on the basis of their location.

By using the RAPD-PCR technique (Kautenburger, 2006) studied the genetic resemblance between Lumbricus terrestris population that were collected from two maize fields. Result showed that the two populations with the similarity index ranging between 0.60 to 0.73. Kautenburger, by using the same technique also studied the genetic diversity in the populations of *L. terrestris* that were collected from five different sampling areas in Germany. The intra and interspecific population showed the similar results with inter and intra population variations of 27.9% and 18.0% respectively (Kautenburger, 2006). Earlier studies suggested that the diverse use of pesticides and rotation of crops have highly influenced the earthworms leading to genetic changes (Brooks *et al.,* 1992; Pfiffner and Mader, 1997; Blakemore, 2000; Kautenburger, 2006; Dyer *et al.,* 1998). (Lentzsch and Golldack 2006) first applied, RAPD technique to *L. terrestris* and *Aporrectodea* spp. respectively. (Meenatchi *et al.*, 2009) used RAPD-PCR technique to examine genetic variations in 6 strain of *Eudrilus eugeniae* (Kinberg) sampled from six sites in three different states of south India by making use of 20 random primers. The results showed difference in the genetic profiling. The similarity matrix ranged between 0.40-0.90. By using symmetric matrix for different strains (Meenatchi *et al.,* 2009) constructed a dendogram that resulted into two major clusters. Cluster 1 comprised of individuals that belong to four locations namely Bijapur Dharwad, Raichur and Nagpur while the second one consisted of the individuals belonging to Coimbatore and Bangalore strains. They also pooled the samples and the results showed maximum genetic similarity of 1.0 and 0.90 between Bijapur and Dharwad strains, Bijapur and Nagpur strains of Karnataka state respectively. A similarity coefficient of 0.71 was reported between Dharwad and Raichur, Bijapur and Raichur strains.

The lowest genetic resemblance was experienced among Nagpur and Bangalore strains and Nagpur and Coimbatore strains with the genetic similarity value of 0.40. (Biruntha *et al.,* 2013) used RAPD in Perionyx excavatus sampled from four different sites to analyze the extent of genetic variation and polymorphism. The results obtained from these samples suggested that the specimen collected from Sirumalai Hills and Dindigul have more taxonomical similarity than those collected from Vadipatti.

CONCLUSION

In the recent times molecular techniques have evolved out to be the effective and precise tool that have established themselves to be valuable in overcoming the limitations of the conventional methods in manuscripting the existing earthworm diversity.

The study concluded: (i) earthworm abundance and distribution depends on a number of factors viz., soil and climatic parameters and crop management practices; (ii) density and biomass of the earthworms is directly related to the annual rainfall and inversely proportional to soil texture;(iii) integrated approach of taxonomy may be helpful to record larger endemic beneficial species of earthworms however, molecular markers may be an important tool for the classification and examination of diversity of earthworms.

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