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RESEARCH ARTICLE

KARYOLOGICAL STUDIES ON TWO SPECIES OF EARTHWORM OF GENUS *Metaphire* FROM JAMMU REGION

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ABSTRACT

Karyological studies on two earthworm species ie., *Metaphire posthuma* and *M. houletti* from Jammu region have been carried out during the present investigation. The diploid chromosome number 2n=28 has been observed for both the species. Various morphometric parameters were also studied.

INTRODUCTION

Earthworms form a major component of the soil fauna. They are known to inhabit the earth for the last 600 million years and silently working day and night in improving the quality of our soil. Earthworms are also involved in the production of an excellent fertilizer, called as vermicompost and these are also used as a source of animal protein for domesticated animals. For these and other reasons earthworms are worthy of investigation.

Although there is a good interest in earthworms by ecologists and the use of molecular markers in ecological research is growing over the past 20 years, molecular ecology which embraces778 topics as varied as population genetics, molecular evolution, behavioural ecology and biogeography (Freeland, 2005) is a poorly developed area of research in earthworms.

Relatively few molecular analysis tools have been developed for earthworms despite the economic and ecological importance of these soil ecosystem engineers (Kautenburger, 2006). This is due to lack of interest in taxonomy of earthworms among earthworm workers as the identification of earthworms is very complex because it involves proper preservation of specimens and study of internal structures. Thus, a proper understanding for identification of earthworms, including those used in vermiculture and vermicomposting is urgently needed.

In earthworms some anatomical and morphological characters commonly used in taxonomy usually coincide within different taxa (Pop *et al*., 2003). These limitations have led similar species that are thought to be phylogenetically closely related, included in a species complex but within an unknown status (Sims and Gerard, 1991; Briones, 1993, 1996). So, in case of earhworms problematic taxonomy and the variable level of ploidy within and among species are significant barriers to research on population genetics.

In taxonomy, cytological data such as chromosome number and position of centromere are regarded as more significant than other taxonomic evidences. So, karyological study will help in studying the systematic in case of earthworms. The aim of present investigation is to study the karyotypes of earthworm species *Metaphire posthuma* and *M. houletti*.

MATERIAL AND METHODS

The specimens were collected from the soil from the soil by digging method from different locations of District- Jammu. Most of the collection was done during the rainy season.

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For the chromosomal study, an adult earthworm was taken and pretreated with 0.05% of colchicine solution which was injected in between the head and the clitellum of selected worm at a dosage of 0.1 ml per 1gm body weight and left for 24 hours. After the pretreatment the earthworm was dissected and internal organs i.e., seminal vesicles, testis and tail were taken out and transferred to 0.075% hypotonic KCl solution and kept for 80-90 minutes.The tissues after hypotonic treatment were fixed in freshly prepared Cornoy's fixative (3:1 methyl alcohol and glacial acetic acid) and kept for 20 minutes giving 3 changes of fresh fixative. Slides were prepared by dabbing technique using air drying method following the techniques of Carr and Walker (1961) and Mehlop and Gardner (1982) with slight modifications. The slides were stained with 2% Giemsa Stain, later rinsed with distilled water and air dried.

Scanning and photomicrography of the slides was done using Olympus CH20iBIMF binocular research microscope and SSC-DC378P camera respectively. Well spread suitable mitotic and meiotic stages were photomicrographed at a magnification of 100x.

RESULTS

During the present study, chromosomes of two species of earthworms viz., *M. posthuma* and *M. houletti* belonging to the family Megascolecidae were investigated.

Metaphire posthuma

The observed diploid number was 28 in *M. posthuma*. The karyotype (Fig.2) was prepared from well spread metaphase complement (Fig.1) which shows the presence of 28 elements, all were biarmed and showed gradation in size. Histogram (Fig.5) was prepared on the basis of decreasing value of RL% from chromosome pair one to fourteen. While idiogram (Fig.6) was prepared on the basis of decreasing length of chromosome pair one to fourteen. The karyotype revealed the presence of 2 pairs of metacentrics, 7 pairs of submetacentrics, 3 pairs subtelocentrics and 2 pairs of telocentrics. Thus, the chromosomal formula was determined as 2m+7sm+3st+2t. The morphometric data of the karyotype is tabulated in table 1.

Actual mean length of the largest chromosome - 2.23µm
Actual mean length of the smallest chromosome - 0.60µm
Relative length percentage of the largest chromosome - 100%
Relative length percentage of the smallest chromosome - 26.90%
Ratio of the largest to the smallest chromosome - 3.71
Total diploid length - 39.84µm
Fundamental arm number = 52

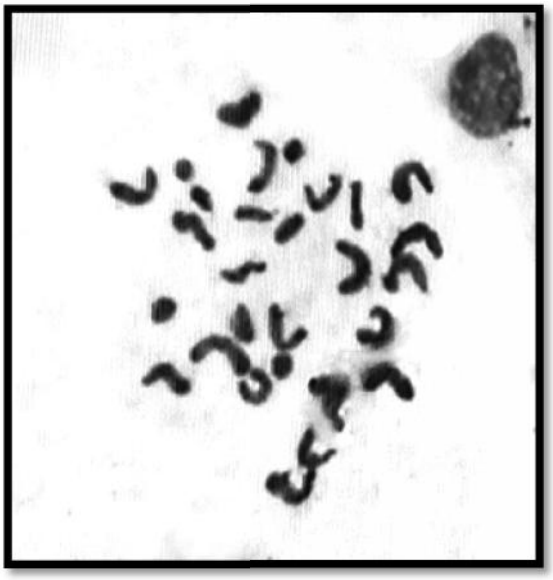

Fig. 1 Spermatogonial metaphase complement of *Metaphire posthuma* (2n=28)

Metaphire houletti

The diploid number recorded from the somatic metaphase complement was 28. The karyotype (Fig. 4) was prepared from well spread metaphase complement (Fig.3) which shows the presence of 28 elements, all were biarmed and showed gradation in size. Histogram (Fig.7) was prepared on the basis of decreasing value of RL% from chromosome pair one to fourteen. While idiogram (Fig.8) was prepared on the basis of decreasing length of chromosome pair one to fourteen. The karyotype revealed the presence of 9 pairs of metacentrics, 3 pairs of submetacentrics, 2 pairs subtelocentrics.

Table 1 Morphometric data of karyotype of *Metaphire posthuma* (2n=28)

Chromosome Pair Number	Mean Length of Short Arm (p) in µm	Mean Length of Long Arm (q) in µm	Absolute Length (p+q) of the Chromosome	Arm Ratio (q/p)	Relative Length Percentage	Total Complement Length Percentage	Centromeric Index	Nomenclature
1	0.82	1.41	2.23	1.71	100	5.59	36.77	Submetacentric
2	0.59	1.46	2.05	2.47	91.92	5.14	28.78	Submetacentric
3	0.68	1.26	1.94	1.85	86.99	4.86	35.05	Submetacentric
4	0.62	1.29	1.91	2.08	85.65	4.79	32.46	Submetacentric
5	0.61	1.22	1.83	2.00	82.06	4.59	33.33	Submetecentric
6	0.62	1.04	1.66	1.67	74.43	4.16	37.34	Metacentric
7	0.39	1.24	1.63	3.17	73.06	4.09	23.92	Subtelocentric
8	0.44	1.05	1.49	2.38	66.81	3.73	29.53	Submetacentric
9	0.34	0.77	1.11	2.26	49.77	2.78	30.63	Submetacentric
10	0.48	0.56	1.04	1.16	46.63	2.61	46.15	Metacentric
11	0.22	0.72	0.94	3.27	42.15	2.35	23.40	Subtelocentric
12	0.17	0.65	0.82	3.82	36.77	2.05	20.73	Subtelocentric
13	0.07	0.60	0.67	8.57	30.04	1.68	10.44	Telocentric
14	0.06	0.54	0.60	9.00	26.90	1.50	10.00	Telocentric

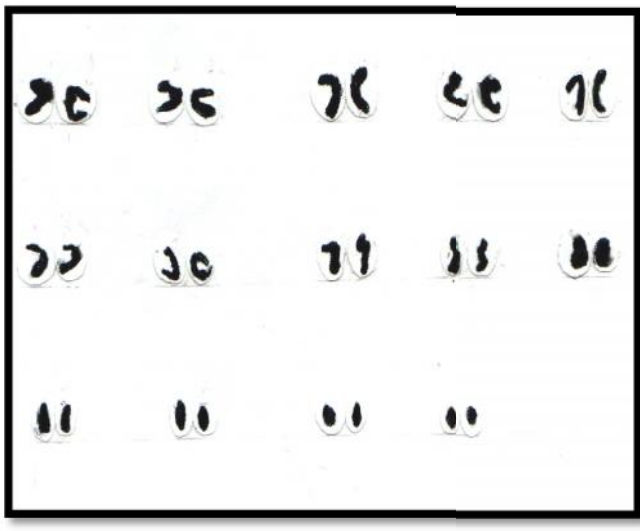

Fig. 2 Karyotype of metaphase complement of *Metapire posthuma* showing 2n=28 (2m+7sm+3st+2t)

Fig. 3 Somatic metaphase complement of *Metaphire houletti* (2n=28)

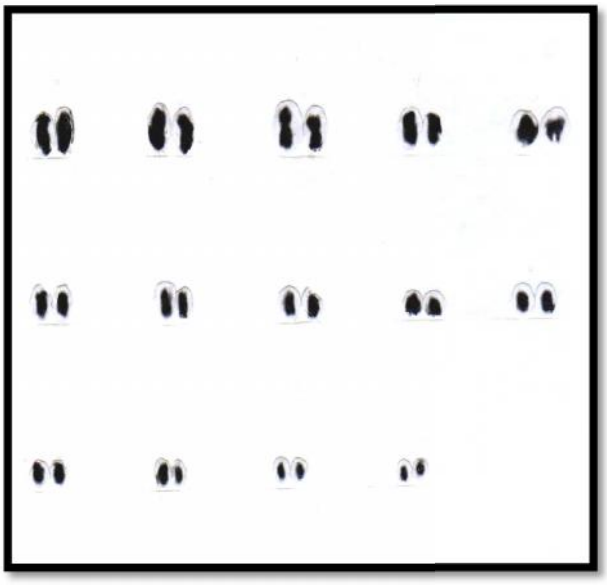

Fig. 4 Karyotype of metaphase complement of *Metapire houletti* showing 2n=28 (9m+3sm+2st).

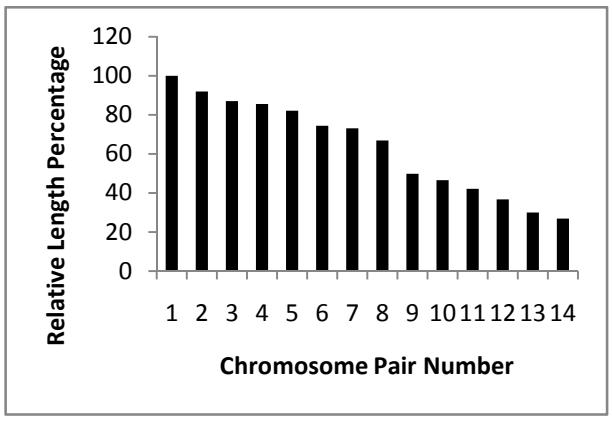

Fig. 5 Histogram of *Metaphire posthuma*

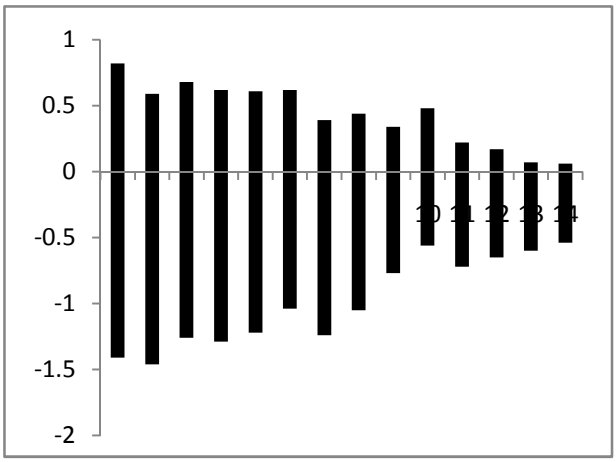

Fig. 6 Idiogram of *Metaphire posthuma*

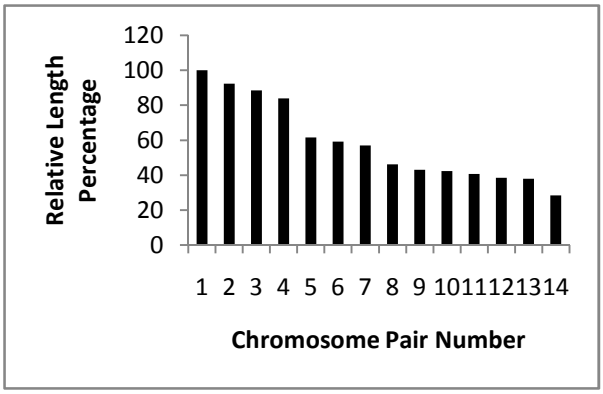

Fig. 7 Histogram of *Metaphire houletti*

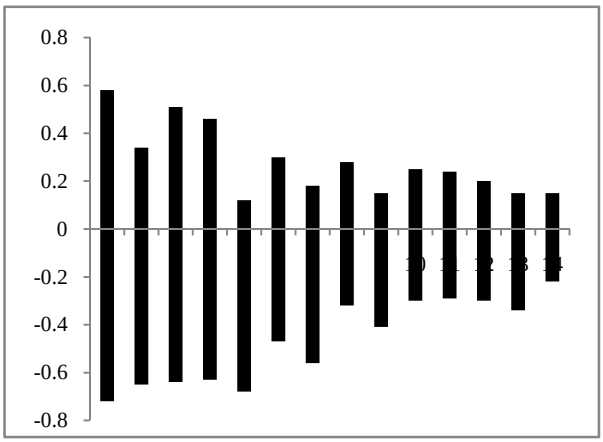

Fig. 8 Idiogram of *Metaphire houletti*

Table 2 Morphometric data of karyotype of *Metaphire houletti* (2n=28)

Chromosome Pair Number	Mean Length of Short Arm (p) in µm	Mean Length of Long Arm (q) in µm	Absolute Length (p+q) of the Chromosome	Arm Ratio (q/p)	Relative Length Percentage	Total Complement Length Percentage	Centromeric Index	Nomenclature
1	0.58	0.72	1.30	1.24	100	6.10	44.61	Metacentric
2	0.34	0.65	1.20	1.91	92.30	5.63	28.33	Submetacentric
3	0.51	0.64	1.15	1.25	88.46	5.39	44.34	Metacenric
4	0.46	0.63	1.09	1.36	83.84	5.11	42.20	Metacentric
5	0.12	0.68	0.80	5.66	61.53	3.75	15.00	Subtelocentric
6	0.30	0.47	0.77	1.56	59.23	3.61	38.96	Metacentric
7	0.18	0.56	0.74	3.11	56.92	3.47	24.32	Subtelocentric
8	0.28	0.32	0.60	1.14	46.15	2.81	46.66	Metacentric
9	0.15	0.41	0.56	2.73	43.07	2.62	26.78	Submetacentric
10	0.25	0.30	0.55	1.20	42.30	2.58	45.45	Metacentric
11	0.24	0.29	0.53	1.20	40.76	2.98	45.28	Metacentric
12	0.20	0.30	0.50	1.50	38.46	2.34	40.00	Metacentric
13	0.15	0.34	0.49	2.26	37.98	2.30	30.61	Submetacentic
14	0.15	0.22	0.37	1.46	28.48	1.73	40.54	Metacentric

Thus, the chromosomal formula was determined as 9m+3sm+2st. The morphometric data of the karyotype is tabulated in table 2.

Actual mean length of the largest chromosome - 1.30µm
Actual mean length of the smallest chromosome - 0.37µm
Relative length percentage of the largest chromosome - 100%
Relative length percentage of the smallest chromosome - 28.46%
Ratio of the largest to the smallest chromosome - 3.51
Total diploid length - 21.3µm
Fundamental arm number = 56

DISCUSSION

Metaphire is an Asiatic genus which is a member of the pheretima complex, a speciose group of more than 800 described species within 12 genera belonging to the megascolecidae (Blackmore, 2002; Easton, 1979, 1982; James, 2005a, b; Sims and Easton, 1972).

For the family Megascolecidae chromosomal information is available for a few species. Murchie (1967) observed 11 species of the genus *Diplocardia* and this genus is known to have about 30 species.the recorded haploid number is 22 for the diplocardian earthworms. Another species *Amynthas catenus* was studied karyologically by Shen *et al*., 2011 and the haploid number 56 was assigned to it. In case of earthworms the available information is usually limited to the basic chromosome numbers and a level of ploidy and nearly everywhere it doesnot include the statistical measurements of chromosomes (Vitturi *et al*., 1991).

Both the species viz., *M. posthuma* and *M. houletti* were investigated kayologically for the first time during the present study. Both were found to have the same diploid number 2n=28 and thus can be considered to be cytologically similar.

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BIBLIOGRAPHY

Blakemore, R.J. 2002. Cosmopolitan Earthworms—An Eco-Taxonomic Guide to the Peregrine Species of the World. (First CD ed.). VermEcology, Australia.

Briones, M.J.I. 1993. Two ecotypes in *Allolobophora caliginosa* (Oligochaeta, Lumbricidae). Acta Oecologica, 14**:** 317–325.

Briones, M.J.I. 1996. A taxonomic study of the *Allolobophora caliginosa* complex, Oligochaeta, Lumbricidae: a preliminary study. C. J. Zoolog., 74: 240–244.

Carr, D.H. and Walker, J.E. 1961. Carbol fuchsin as a stain for human chromosomes. Stain Tech., 36: 233-236.

Easton, E.G. 1979. A revision of the ‘‘acaecate” earthworms of the Pheretima group (Megascolecidae: Oligochaeta): *Archipheretima*, *Metapheretima*, *Planapheretima*, *Pleionogaster*, and *Polypheretima*. Bull. Br. Mus. Nat. Hist. (Zool.), 35: 1–126.

Easton, E.G. 1982. Australian pheretimoid earthworms (Megascolecidae: Oligochaeta): A synopsis with the description of a new genus and five new species. Aust. J. Zool., 30: 711–735.

Freeland, J.R. 2005. Molecular ecology. Wiley, Chichester.

James, S.W. 2005a. New genera and species of pheretimoid earthworms (Clitellata: Megascolecidae) from southern Luzon, Philippines. Syst. Biodivers., 2: 271–279.

James, S.W. 2005b. Preliminary molecular phylogeny in the Pheretima group of genera (Crassiclitellata: Megascolecidae) using Bayesian analysis. In: Pop, V.V., Pop, A.A. (Eds.), Advances in earthworm taxonomy II (Annelida: Oligochaeta). Cluj University Press, Cluj-Napoca, Romania, pp. 129–142.

Kautenburger, R. 2006. Genetic structure among earthworms (*Lumbricus terrestris* L.) from different sampling sites in western germany based on random amplified polymorphic DNA. Pedobiologia, 50: 257-266.

Murchie, W.R. 1967. Chromosome Numbers of Some Diplocardian Earthworms (Megascolecidae-Oligochaeta). American Midland Naturalist, 78(2): 534-537.

Pop, A.A., Cech, G., Wink, M., Csuzdi, C. and Pop, V.V. 2003. Use of 18S, 16S rDNA and cytochrome c oxidase sequences in the molecular systematics of the earthworm family Lumbricidae (Annelida, Oligochaeta). Pedobiologia, 47: S43-S52.

Shen, H.P., Tsai, C.F., Fang, Y.P. and Chen, J.H. 2011. Parthenogeneis, polyploidy and reproductive seasonality in the Taiwanese mountain earthworm *Amynthas catenus* Tsai *et al.*, 2001 (Oligochaeta, Megascolecidae). Pedobiologia, 54: 133-139.

Sims, R.W. and Easton, E.G. 1972. A numerical revision of the earthworm genus *Pheretima* auct. (Megascolecidae: Oligochaeta) with the recognition of new genera and an appendix on the earthworms collected by the Royal Society North Borneo Expedition. Biol. J. Linnean Soc., 4: 169–268.

Sims, R.W. and Gerard, B.M. 1999. Earthworms. Notes for the identification of british species. London, UK: The Linnean Society and the Estuarine and Coastal Sciences Association.

Vitturi, R., Colombera, D., Catalano, E. and Amico, F.P. 1991. Karyotype analysis, nucleolus organizer regions and C-banding pattern of *Eisenia foetida* (Oligochaeta, Lumbricidae), Genetica, 83: 159-165.

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