Short term somatic cell culture approach for cytogenetic analysis of *Crossopriza lyoni* (Spider: Pholcidae)

Anjali, Sant Prakash

***Abstract*— *Crossopriza lyoni* (Daddy long leg) a typical representative of the family Pholcidae is a spider commonly found in Agra region. The diploid chromosome number of *C.lyoni* (2n=24) with meta and sub-metacentric groups of chromosome were recorded. Two exceptionally large XX is found in female while the males have single X type. The male chromosome was further confirmed through NOR-Ag staining by presence of single Interphasic nuclei in male. The Somatic Cell Culture approach in the current study on *C. lyoni* spider cytogenetic is a convenient technique over the existing practice of repeated sacrifices of spiders and may have various other applications in the field of biotechnology. The present data is also being reported for the first time on *C. lyoni* of Agra region.**

***Index Terms*— Spider, Pholcidae, Cytogenetic, Chromosomes, Somatic cell culture, Agra.**

I. INTRODUCTION

Crossopriza lyoni (Pholcidae) is found across the Indian Subcontinent[1]and exist as a dominant spider group in the semi-arid regions of Agra (26°44´N 27°55´S 77°26´W 78°32´E).Incidentally, the first report of *C. lyoni* was also from Agra region[2] which indicates the robustness of this species to survive in the extreme climate of this habitat. Spiders in general have high ecological, economic and predatory importance; therefore their contribution to agro-ecosystems is well established recently their role in predator controlling mosquito population has also been in focus[3].About 1687 species belong to 438 genera and 60 families have been reported in India[4].The cytogenetical record on pholcids species are scanty and have long been neglected by researchers probably because of its size and also due to the tedious gonadal chromosome preparation techniques[5-6]

It is an accepted fact that the chromosomal information of a species are essential to modern taxonomist and since the species are considered to be the objective reality of some particular genetic continuity. Spiders Cytogenetics has shown a great diversity in diploid chromosome numbers that ranges from 7 to 110[7].The pholcids were known to exhibit 2n ranging from 15 to 34 but their sex chromosome system is not confirmed because of the variations in 2n numbers[8]. In the present study somatic cell culture method has been applied to successfully harvest metaphase plates for Cytogenetical analysis of *C. lyoni* from Agra.

Manuscript received August 04, 2014.

Anjali, Molecular Genetics Laboratory, Department of Zoology, Faculty of Science, Dayalbagh Educational Institute, Agra 282005, India.

Sant Prakash, Molecular Genetics Laboratory, Department of Zoology, Faculty of Science, Dayalbagh Educational Institute, Agra 282005, India.

II. MATERIAL AND METHODS

A. Spider collection:

Pholcids were collected from the agriculture fields of Agra by regular visual searching and Hand collection method[9]

B. Spider Identification:-

Pholcids were identified first through keys and catalogues and confirmed by book records of Indian Spider[10-11]and also through personal communication with Arachnologists.

C. Cell Culture Protocol:-

The protocol was inspired from the existing protocol on water mites as proposed by[12] and little modification on the protocol gave good cell growth.

We have followed two culture media for cell culture for comparison of cell growth and culturing.

1. Drosophila Schneider medium for insects with 10%heat inactivated fetal bovine serum, 2% 1/100 penicillin/ streptomycin (10,000 unit/ml stock), 2% 1/100 glutamine(200 mM stock), 0.35 gmNaHCO3,8.1 ml 2N NaOH and 2.25 g NaCl to which 1000 ml of distil water was added and the final medium was sterilized by filtration. Phytohemagglutinin-M (PHAM) was added to the medium at 2-4 mal per 100 ml.
2. Hikaryol XLTM RPMI culture medium containing L-Glutamine, FBS, PHAM, Penicillin, streptomycin and Sodium bicarbonate.
3. Firstly we autoclave the media according to our set up like for a species s we have taken 10 ml media in two sets or one replicate.
4. 2 ml PHAM was added to 50 ml autoclaved media prepared for other samples also.
5. Spiders were put in a glass or plastic Petri plate and washed with Sterilized water then legs were remove with clean forcep, the body was squashed properly then the body liquid was added to the 5 ml media in sterilized Petri plate mix it slowly. body fluid was depend on the size of the specimen(0.1-0.5 ml)
6. Cell culture was carried out in CO_2 incubator at 28°C for 48-72 hr.
7. A volume of 0.02 ml of Colchicine solution was added to each culture plate and culture was incubated for another 15-20 minutes.

Note- Modification part in our protocol a/c to the culture setup

a) Two culture media were tried initially both shows cell growth but Drosophila Schneider medium was more effective.

b) Cell culture timing was increased because at 28 hrs treatment chromosomes were not properly visualized in shape.

c) At 72 hrs the best metaphase plates were analyzed because the cell growth was very good at this stage according to our observation.

d) The colchicine treatment was decreased (0.02 ml to 0.1 ml) because at high volume the chromosomes were shrunk and not properly visualized.

Chromosomes Preparation:-

1. The culture was transferred to centrifuge tube and spun at 2000 rpm for 5 min.
2. Supernatant was removed and cell pellet was resuspended in 0.075M KCL solution.
3. The cell suspension was incubated at 28°C for 10 minutes and spun at 2000 rpm for 5 minutes after which the supernatant was removed.
4. Cell pellet was agitated and 5 ml of fresh ice cold fixative (1:3 acetic acid: methanol) was added drop by drop. Allowing to stand 10 min at 4°C fixative solution was changed two times using centrifugation
5. The cell pellet was resuspended in 1 ml of fresh fixative and cells were mixed by dropper and dropped on to clean chilled slides which was allowed to air dry and flame dry.
6. Prepared slides were dried overnight and stained with 5% Giemsa solution in Sorensen's buffer, (pH6.8), for 30-40 minutes.
7. Prepared slide were inspected using Olympus microscope and photographs were taken on oil immersion objective at 100X.
8. Best metaphase was photographed.

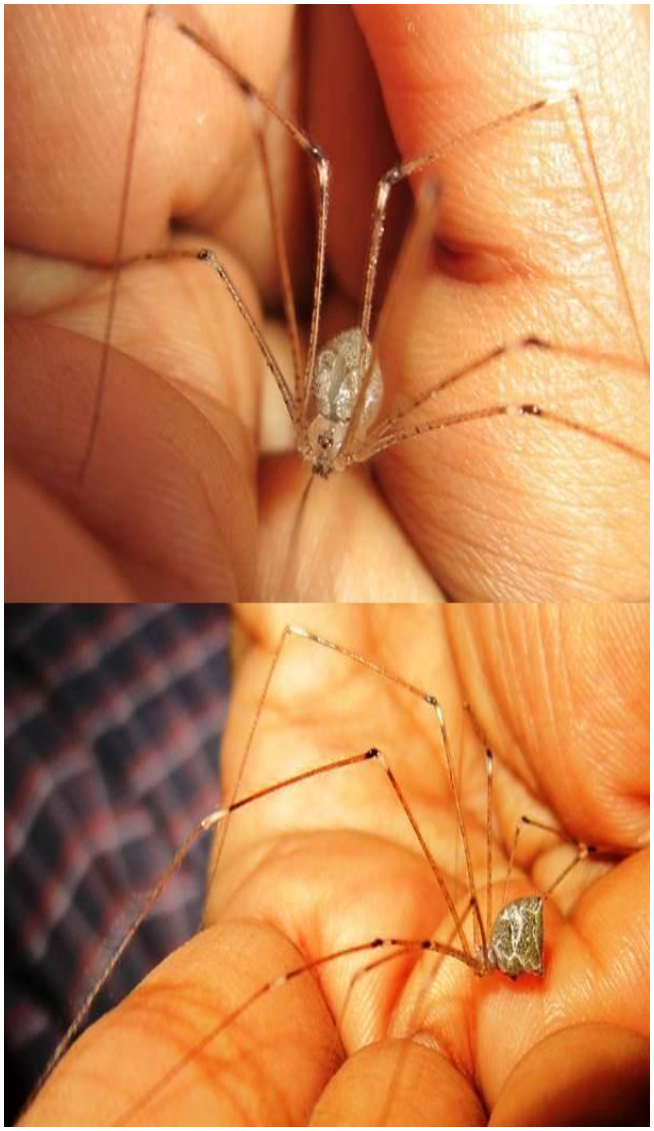


Fig.1*Crossopriza lyoni* (Daddy long spider)

Nucleolar Organizing Regions (NORs)

The one step method of silver staining was used[13].The slides were washed in deionized water several times in order to remove excess stain and dried.[14]

III. Results

C.lyoni is the predominantly abundant spider of Agra region, highly adaptive to semi-arid habitat. The karyological analysis established 2n =24 in female [22+XX] (Fig.2). Total chromosome length (TLC-0.9 cm) (Fig. 3) and the sex chromosome XX was the largest pair among the karyotypes. Karyotype can be categorized under two groups 10 metacentric and 14 sub-metacentric type of chromosome were observed (Fig.4). A single Interphasic NOR stained nuclei was observed in male C. *lyoni* (Fig 5a) while micronuclei were only found in females (Fig 5b).

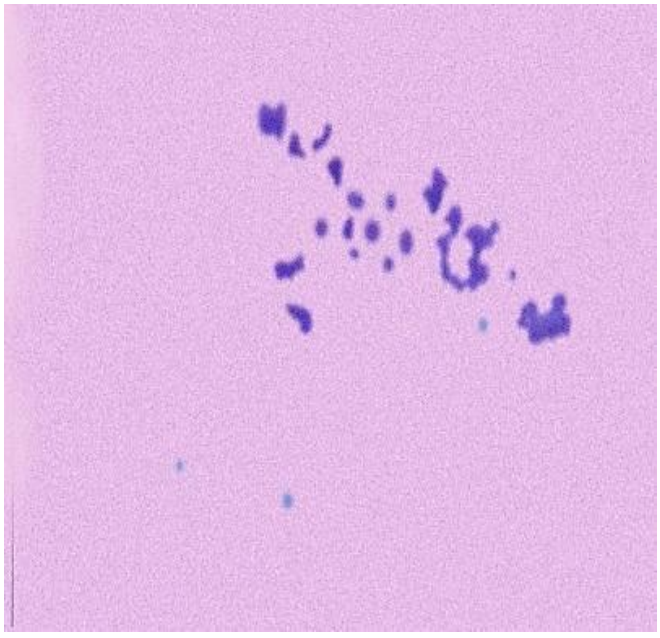


Fig.2 Metaphase Plate of *C. lyoni*

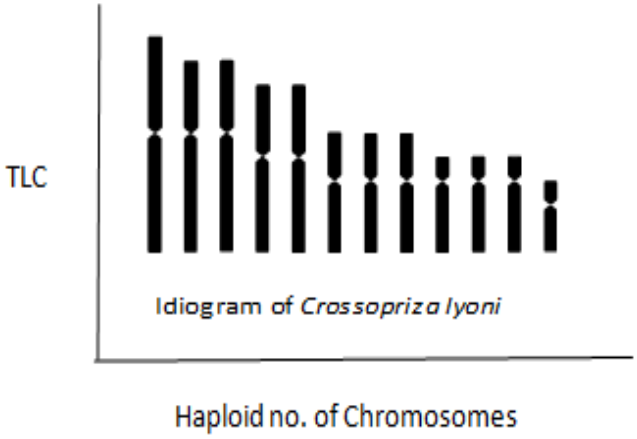


Fig.3 Ideogram of *C. lyoni*

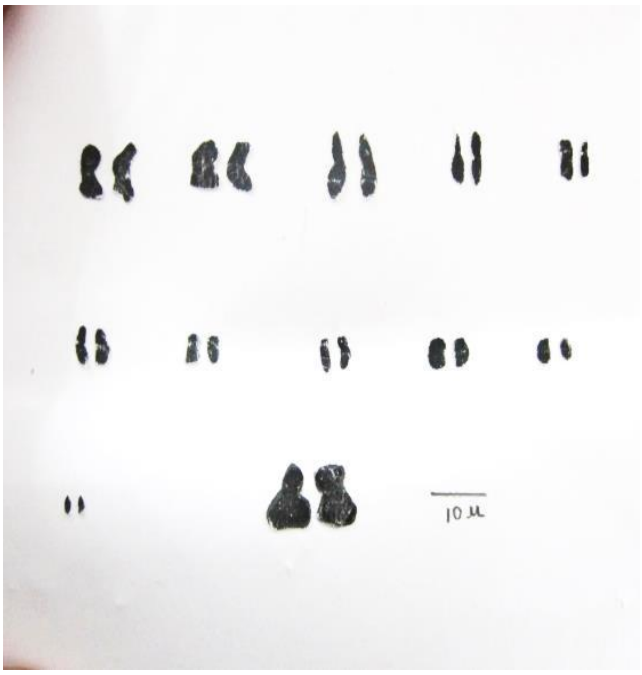
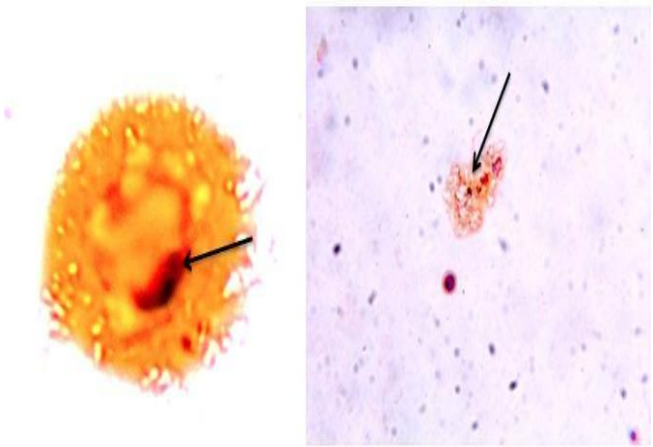


Fig 4 Karyotype of *C. lyoni*



a) Male *Crossopriza lyoni* showing single Interphasic nuclei, b) NOR-Ag stained female micronuclei.

Fig 5a) NOR-Ag staining in *C. lyoni* single Interphasic nuclei, 5b) silver stained female micronuclei.

IV. Discussion

C.lyoni identification was followed by various keys provided by[15, 16, 17]. It is also known as *Pholcus lyoni* commonly known as Box and Daddy long spider females are larger than males their nature is cosmopolitan six pearly white eyes are located at the tip of the cephalothorax, black spots were found all over the legs. Abdomen is grayish off white with black and with patches on sides. A small conical extension is projected at the upper posterior end of the abdomen (Fig. 1)

The use of chromosomal data in Phylogenetic analysis is relatively new, the criteria to codify has not been establish but its use is increasing continuously for Phylogenetic studies. A few studies on Pholcids species exhibit peculiarly metacentric chromosome morphology along with X/XX sex determining chromosome system.

Presently 14 pholcids are cytogenetically known which less than 3% of the total group. Cytogenetic variation was observed in various studies of ***C. lyoni*** 2n =27=26+X[18],2n=25=24+X[19],2n=24=22+X 1X 2n[20]and2n =23=22+X[21] *C. lyoni* exhibits only metacentric and sub-metacentric morphology in all chromosomes component, with a diploid no. 2n= 24(22+XX)[22].Higher numbers of chromosomes have been reported in a few morphological derived species (Mygalonomorphs) with acrocentric chromosomes morphology. According to such characteristics are considered as ancestor[8]. While the metacentric and sub-metacentric chromosome and XX and X system is relatively newer as they may have been derived by the centric fusion followed or not by pericentric inversion by tandem fusions. The presence of $X_1X_2X_3$ type of sex determination system in spiders is a further advancement to the ancestor type[23.] The presence of XX chromosome can visually identified because of hetropycnotic nature. This differences also get highlighted by the NOR-Ag staining[8], an interesting phenomena has been observed in cells of male chromosomes where a characteristic chromatin block is found (Fig.5a) while in female micronuclei were stained (Fig.5b). This provides an easy method in understanding the sex determining mechanism in *C.lyoni*.

The applications of Somatic cell culture method have been to an advantage as the number of species sacrificed in this study was minimum. The harvesting of metaphase plates in required time was also convenient. As the dissection of spiders and removal of gonads is very difficult and the rate of preparation of slides is very slow, the somatic cell culture method helps in overcoming this problem.

The aim of this study was to introduce an innovative method for short term Somatic cell culture for Cytogenetical analysis of chromosomes of spiders of group pholcids and also to unravel specifically the Cytogenetical information of the Pholcids species *C. lyoni.*

Acknowledgment

We are thankful to Dr. Manju Siliwal for her comments on identification of Spiders and we are also thankful to UGC (RGNFS) for providing financial support to Miss Anjali.

REFERENCES

[1] B.K. Tikader, "Handbook of Indian Spiders." *Edited by ZSI* (1987) 251.

[2] J. Blackwall, "Description of several species of East-Indian spiders apparently new or little known to arachnologists." *Annals and Magazine of natural history* ,11 (1867) 393.

[3] Anjali and S. Prakash, "Species Richness and Cytogenetical analysis of Spiders of Agra." International Conference on Entomology, Punjabi university (2014).

[4] S. Keswani, P. Hadole, A. Rajoria, "Checklist of spiders (Arachnida:Araneae) from India." *Indian Journal of Arachnology* 1 (2012) 1.

[5] G. C. Webb, M.J.D. White, Contreras and J. Cheney "Cytogentics of the parthenogenetic grasshopper *Warramaba* (Formerly *Moraba*) *virgo* and its bisexual relatives.IV. chromosomes banding studies." *Chromosoma* 67(1978) 309.

[6] D. Araujo, A.D. Brescovit, C.A. Rheims, D.M. Cella, "Chromosomal data of two pholcids (Araneae, Pholcidae): a new diploid number and the first cytogenetical record for the New World clade." *J Arachnol* 33 (2005) 591.

[7] S. Suzuki, "Cytological studies in spiders III Studies on the chromosome of fifty seven species of spiders belonging to seventeen families with general considerations on chromosomal evolution." *Journal of Hiroshima University* 1 (1954)124.

[8] R.S. Oliveira, A.C. Jesus, A.D. Brescovit and D.M.A. Cella, "Chromosomes of *Crossopriza lyoni* (Blackwall 1867), Intra individual numerical chromosome variation in *Physocyclus globosus* (Taczanowski 1874), and the distribution pattern of NORs (Araneomorphae, Haplogynae, Pholcidae)." *Journal of Arachnology* 35 (2007) 293.

[9] Anjali and S. Prakash, "Diversity of spiders from Semi-Arid habitat of Agra." *Indian Journal of Arachnology* 1(2012) 66.

[10] P.A. Sebastian and K.V. Peter, "Spiders of India." *University press private limited* ISBN:9788373716416 (2009).

[11] N.I. Platnick, "World Spider Catalog, Version 13.5." *American Museum of Natural History*, online at http://research.amnh.org/iz/spiders (2013).

[12] S.T. Onrat, F. Asci and M.A. Ozkan, "Cytogenetics study of *Hydrodromadespiciens* (Muller, 1776) (Acari: Hydrachnellae: Hydrodromidae)." *Genetics and Molecular Research* 5 (2006) 342.

[13] W.M. Howell, D.A. Black, "Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: A 1-step method." *Experientia* 36 (1980) 1014.

[14] S. Prakash, "Nuclear Organizer regions (NORS) localization in the karyotype of the streaked frog, *Rana limnocharis*." *JAdvZool* 16 (1996) 48.

[15] B.J. Kaston , "How to know spiders? The pictured key Nature series." *Wm. C.Brown. Co. Publishers.Dubuque, Iowa* USA (1978) 1272.

[16] R. Preston-Mafham and K. Preston-Mafham, "Spiders of the World, "*Blandford Press Ltd. Dorset, UK* (1984)191

[17] M. Siliwal, M. Sanjay, B.B.K. Biswas, "Indian spiders (Arachnida: Araneae): Updated checklist." *Zoos' Print Journal*20(2005)1999

[18] B. N. Bole –Gowda, "A study of the chromosomes during meiosis in twenty two species of Indian spiders." *Proceedings the Zoological Society of Bengal* 11(1958) 69

[19] M.D.L. Srivastava and S. Shukla, "Chromosome number and sex determining mechanism in forty seven species of Indian spiders." *Chromosome Information Service* 41(1986) 23.

[20] B.B. Parida and N.N. Sharma, " Chromosome number, Sex mechanism and Genome size in 27 species of Indian spiders." *Chromosome information service* 43(1987) 11.

[21] D.P. Sharma and B.B. Parida , "Study of Chromosomes in spiders from Orissa." *Pranikee* 8(197)71.

[22] *Levan, F. Fredga and A.A. Sanberg, "Nomenclature for centromeric position on chromosome." Heredity 52 (1964) 201.*

[23] D. Araujo, D.M. Cell, E. Paul-Neto, C. Schneider, "Sex Chromosomes and Meiosis in spiders:A Review." *Meiosis-Molecular Mechanism and Cytogenetic* Diversity 5(2012)87.