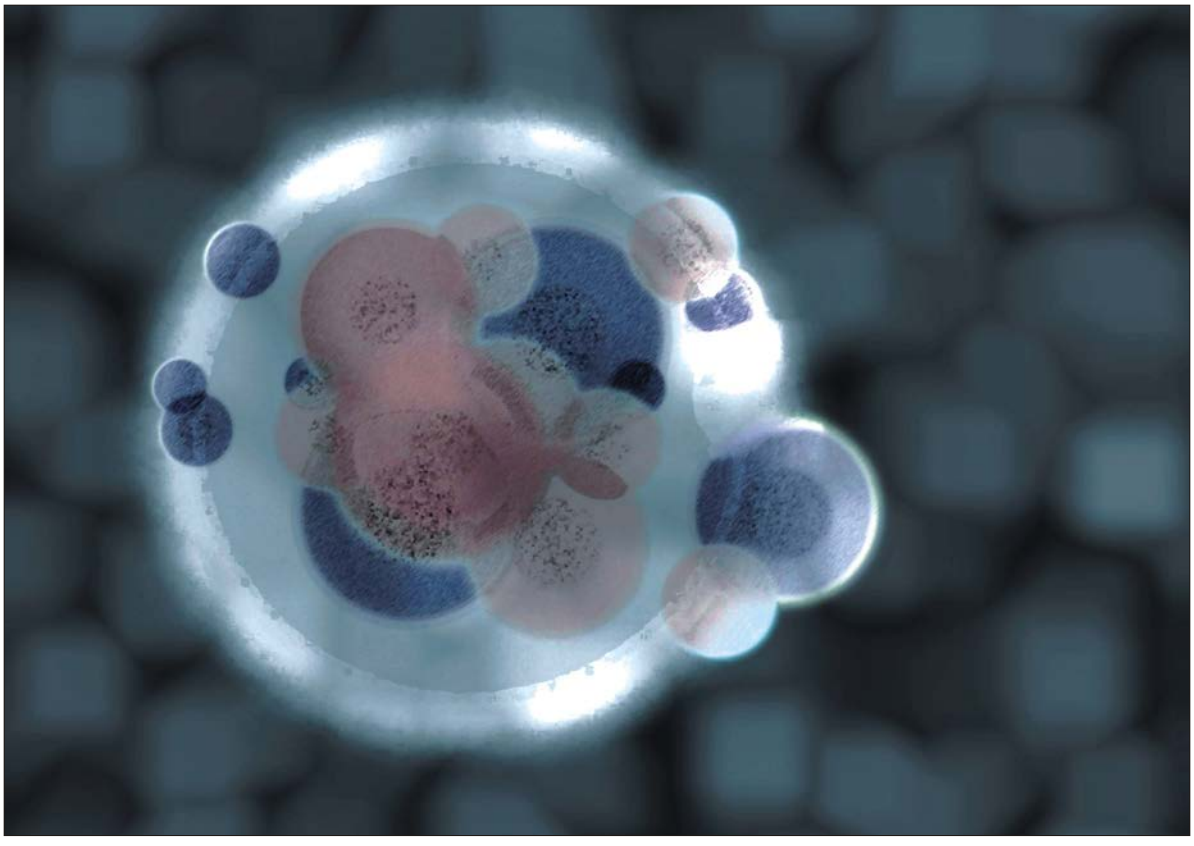

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Development of Karyotype and Localization of Cytogenetic Markers in Dimua River Prawn, *Macrobrachium villosimanus* (Tiwari, 1949)

[1]Nidhi Choudhary, [2]Rupam Sharma, [1]Sumedha Asthana, [1]Pratik Vyas, [2]Mohd Ashraf Rather, [2]A.K. Reddy and [2]Gopal Krishna
[1]Department of Biotechnology and Bioinformatics, C.B.D. Belapur, Navi Mumbai-400 614, Maharashtra, India
[2]Department of Fish Genetics and Biotechnology, Central Institute of Fisheries Education, Versova, Mumbai-400 061, India

Abstract: Karyotype and cytological characteristics of the Dimua river prawn, *Macrobrachium villosimanus* were investigated. The cytogenetic analysis revealed that the diploid chromosome number in this species is 2n = 124 with karyotype formula 2n = 11M+11SM+40T and ST. Sex chromosomes were cytologically indistinguishable. The fundamental arm number was found to be NF = 84. Cytogenetic markers in the form of C bands, G bands and NOR bands were generated. NOR bands were found on the constriction regions near centromeric, as well as, slightly away from terminal regions. While, G bands was at AT-rich regions appearing to be away from centromere towards the end of the chromosome bodies. C bands were localized on the regions adjacent or immediate to the centromeres and on the constrictions as indicating the concurrency with constitutive hetrochromatin.

Key words: Cytogenetic marker, banding, centromeric, diploid chromosome

INTRODUCTION

The freshwater prawn which are mainly from the genus *Macrobrachium* is identified by its enlarge second pair of pereopods (Bate, 1968). There are 193 species of *Macrobrachium* reported from various parts of the world, of which 30 species are found in India (Holthius, 1980; Tiwari and Holthuis, 1996). *Macrobrachium villosimanus* (Tiwari, 1949) is a natural inhabitant of the Brahmaputra River basin of North East region of India. This prawn is commonly called as Dimua river prawn. It is also found in West Bengal and Hooghly Matla Estuary of India. This species belongs to the Palaemonidae family of the crustaceans. Its body colour is translucent and the dorsal side of flagella is brownish red. In rostrum there are 12-13 teeth on upper part and 8-9 teeth on lower part. It grows upto 10.6 g in 6 months. The species will contribute to the species diversification in aquaculture.

Cytogenetics plays a very major role in the discovery of markers. The chromosome number and the morphology are being used as cytogenetic marker of different species. These markers also provide information about the phylogeny evolution and taxonomic relationship. Cytogentic markers are also widely used for genetic improvement programs. Karyotype is an organized profile of a chromosomal set which describes the number and characteristics of the chromosomes. Knowledge about chromosome and karyotype helps in genetic characterization of the species and also find out the level of introgression or hybridization if any with the population. It forms the basis of genetic improvement program and conservation of wild gene pool. Sharma *et al.* (1960) were pioneer in cytogentical studies in India. Following this Indian researchers has carried out such in more than 125 species from both freshwater and marine water (Rishi, 1989). Each chromosome pair normally possesses distinct "barcode" of bands which cannot be viewed without special treatment to the chromosomes. Hence, banding techniques are applied which generate a characteristic banding pattern of the chromosomes. Although *M. villosimanus* seems to be an alternative candidate species for aquaculture, there are very less references on the species. So far there is no record of cytogenetical study on *Macrobrachium villosimanus*. This study could detail the information pertaining to chromosome number and karyotype. Hence present study was carried out on *Macrobrachium villosimanus* for characterization using karyotyping and cytogenetic markers such as C-,(centromeres) G- (Giemsa) and NOR(nucleolar organizing region) bands. This is the

Corresponding Author: Rupam Sharma, Department of Fish Genetics and Biotechnology, Central Institute of Fisheries Education, Versova, Mumbai, 400 061, India Fax: +91 22 26361575

probably first report on Karyotype of *Macrobrachium villosimanus* which forms the basis of the chromosomal studies.

MATERIALS AND METHODS

Chemicals such as Colchicine and Geimsa stain (Himedia), Glacial acetic acid, Sodium citrate solution, Carnoys fixative were used for slide preparation. For banding techniques, chemicals used were of reagent grade such as Hydrochloric acid, Barium hydroxide, 2XSSC (Saline Sodium Citrate), Trypsin, Silver Nitrate, Colloidal developer (Gelatin and pure Formic acid) that were procured from local vendors. All other chemicals were of reagent grade.

Live specimens of *M. villosimanus* were collected from Brahmaputra River in Assam, India and were maintained in the hatchery of Central Institute of Fisheries Education, Versova, Mumbai, India. Cell division was arrested at metaphase by the dip treatment method using 0.05% Colchicine solution at the rate of 1 mL 100 g^{-1}. Twenty specimens were used for the same. The prawns were kept in a well aerated container containing the solution for 3 h prior to tissue extraction. The prawns were then dissected and the whole body tissue was taken which were placed in the hypotonic solution (0.9% sodium citrate), for 45 min. The tissues were chopped for uniform absorption of hypotonic solution by all the tissues. The chopped tissue was then transferred to Carnoys fixative (Methanol (3): Glacial Acetic Acid (1)), by changing the solution at regular interval of 15 minutes for 3 times. The tissue was stored in refrigerator overnight. For slide preparation, the stored tissue was thawed and a tissue suspension was prepared in 45% acetic acid by gentle shaking, taking care not to make the solution too turbid which was followed by centrifugation at 3000 rpm for 10 minutes at 27°C. The centrifugation of the solution was repeated for 3-4 times until a clear transparent solution was obtained. The suspension was dropped on the clean and pre-warmed slides from a particular height so that the cells burst open. After air drying, the slides were stained with Geimsa (8%) for 45 min. The stained slides were washed with distilled water to remove the excess stain and allowed to air dry. The prepared slides were observed under a Hund microscope. Complete good quality chromosome spreads were photographed using a digital camera attached to the microscope. About 225 chromosome spreads from 20 specimens were observed to find out the diploid chromosome number of the species.

For karyotype preparation, few chromosome spreads were selected from those 225 spreads and chromosomes were cropped individually. The chromosomes were then classified following Levan *et al*. (1964) and homologous chromosomes were arranged according to their shape viz. Metacentric chromosomes, Sub-Metacentric chromosomes, Telocentric chromosomes and Sub-Telocentric chromosomes.

Banding techniques: Characteristic banding patterns of chromosomes at mitotic metaphase were generated to analyze the structural details. Special staining procedures were performed to obtain a characteristic pattern of chromosomes that permit their identification throughout a species. The banding techniques were followed after allowing the chromosomes to age for specific periods. C-Banding was carried out as described by Sumner (1972); Nucleolar organizing regions were studied through silver staining method/NOR Banding as described by Howell and Black (1980) while G-Banding was developed following the protocol given by Abe and Muramoto (1974) with slight modification.

Statistical methods: All data presented are expressed as Means±standard error with the help of SPSS-16.0 version software.

RESULTS

A well spread mitotic metaphase of chromosomes of *M. villosimanus* was obtained after a number of trial and error methods for standardization. After screening 225 spreads from 20 specimens, the modal diploid chromosome number of *M. villosimanus* was found to be 2n = 124 with the karyotype formula 2n = 11M+11SM+40ST and T. The chromosomes were classified by arranging the homologous pairs starting with metacentric chromosomes, sub-metacentric chromosomes, telocentric chromosomes and sub-telocentric chromosomes. The fundamental number of chromosome arms (NF) has been calculated as 84 by summing twice the number of metacentric and sub-metacentric chromosomes and the number of telocentric and sub-telocentric chromosomes. The detailed result of the karyotype has been presented in Table 1. The chromosome spread and the karyotype has been presented in Fig. 1 and 2, respectively.

Positive cytogenetic markers of C-Bands, G-Bands and NOR-Bands were generated in the metaphase chromosomes spread of *Macrobrachium villosimanus*.

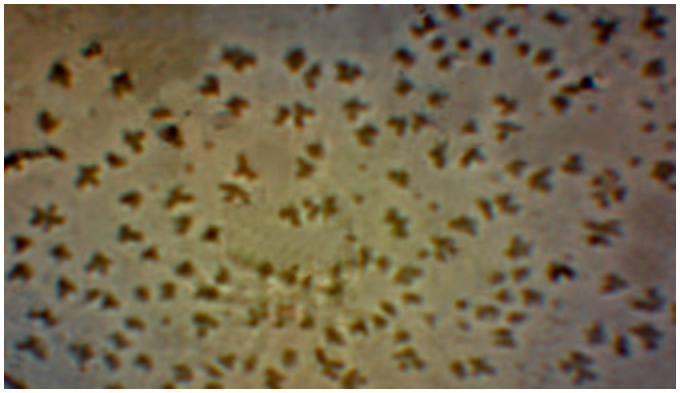


Fig. 1: Metaphase chromosome spread of *Macrobrachium villosimanus*

Table 1: Fundamental arm number (NF) table showing chromosome set analyses

	Chromosome No.					
Animal No.	122	124	126	2n modal no.	NF	Total no. of spread observed
1	-	5	2	124	84	7
2	2	6	1	124	84	9
3	-	9	-	124	84	9
4	2	10	-	124	84	12
5	-	12	2	124	84	14
6	1	8	-	124	84	9
7	1	9	1	124	84	11
8	-	10	-	124	84	10
9	-	13	2	124	84	15
10	6	3	2	122	82	11
11	-	8	-	124	84	8
12	1	1	5	126	86	7
13	-	12	1	124	84	13
14	-	9	-	124	84	9
15	-	15	-	124	84	15
16	-	12	1	124	84	13
17	7	4	3	122	82	14
18	4	8	-	124	84	11
19	1	14	1	124	84	15
20	-	13	-	124	84	13

The chromosome spreads with these bands have been presented in Fig. 3. These bands characterize the chromosomes on the basis of the characteristic structural patterns generated. NOR bands were found on the constriction regions near centromeric, as well as, slightly away from terminal regions. While, G bands was at AT-rich regions appearing to be away from centromere towards the end of the chromosome bodies. C bands were localized on the regions adjacent or immediate to the centromeres and on the constrictions as indicating the concurrency with constitutive hetrochromatin.

DISCUSSION

For preparing a metaphase chromosome spread rapidly growing tissues are used (Tan *et al*., 2004). Classical sectioning or squashing method was used for chromosome studies of decapods (Murofushi *et al*., 1984 and Murofushi and Deguchi, 1990). Later on air drying method was developed which had revolutionized the cytogentic studies. Yet in decapods the cytogentic studies are very scanty. This may be due to the presence of small size and large number of chromosome in these species. Few of the important work that has been reported from decapods are (Chow *et al*., 1990; Justo *et al*., 1991; Murofushi *et al*., 1991; Xiang *et al*., 1994; Tiwari and Holthuis, 1996). There is no report available on the diploid chromosome number of *M. villosimanus*. So to find out this, 200 slides of chromosome spreads out of 20 specimens were prepared and 225 chromosome spreads were screened to find out the modal chromosome number of 124 for this species. It has been found out that there are 11 pairs of metacentric chromosomes, 11 pairs of sub-metacentric chromosomes and 40 pairs of sub-telocentric

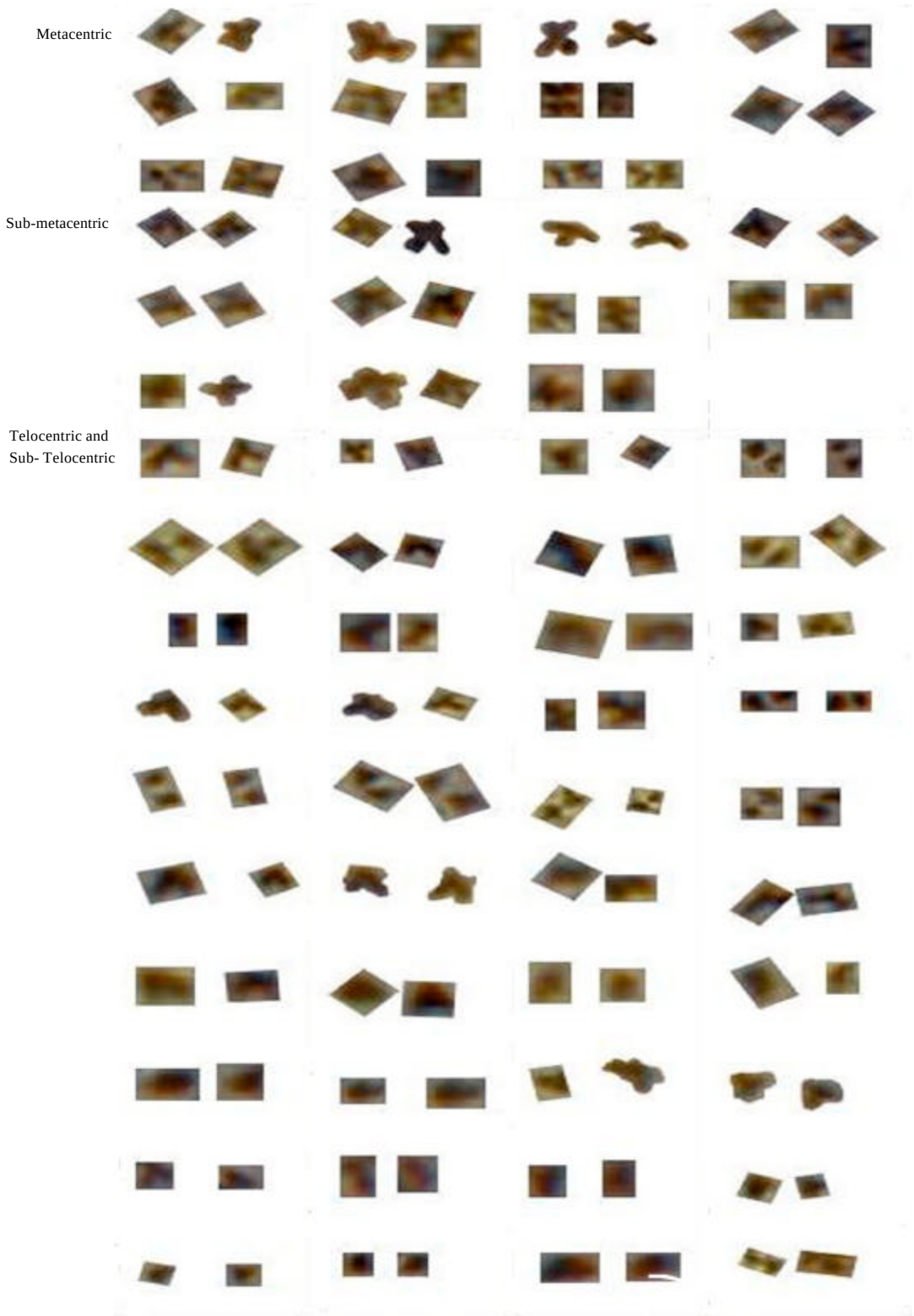


Fig. 2: Karyotype of *Macrobrachium villosimanus*

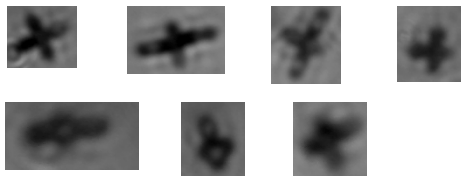
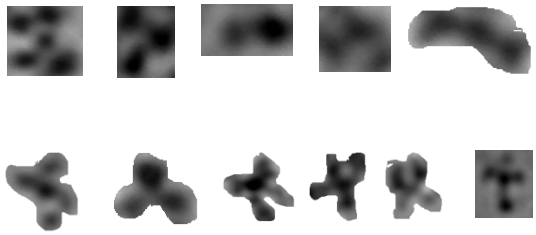
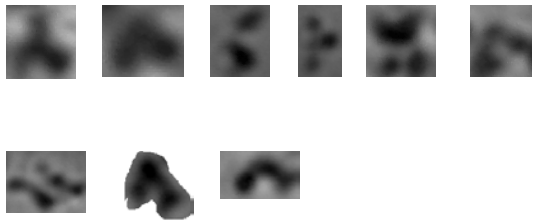
Banding Type	Results
NOR($AgNO_3$) banding	
C-banding	
G-banding	

Fig. 3: Chromosome showing Sliver stained NORs, C-bands and G-bands

and telocentric chromosomes in this species. Since there are no reports on the diploid chromosome number of this species yet, our results cannot be compared and the present finding is the first report on *M. villosimanus*.

However, there are various reports of cytogenetic study on different species of the genus *Macrobrachium* which showed that the diploid chromosome number is always more than 100 except one i.e., *Macrobrachium carcinus* of which the diploid chromosome number is 2n = 94 with the karyotype formula 2n = 94 T as all the chromosomes in this species are telocentric (Indy *et al*., 2009). In *Macrobrachium rosenbergii*, the diploid chromosome number as 2n = 118 with the karyotype formula of 26M+27SM+6ST and T has been reported (Damrongphol *et al*., 1991). A different karyotype formula of *Macrobrachium rosenbergii* with 2n = 118 was also found out which was 2n = 45 M and SM+14T and ST (Justo *et al*., 1991). It has been observed that there is variation in chromosome formula in *M. rosenbergii* even if the total chromosome number is same. The chromosome number of *Macrobrachium nipponense* is 2n = 104 having karyotype formula 2n = 22M+52SM+22T+8ST (Gaofeng *et al*., 1994) whereas *Macrobrachium superbum heller* has 2n=100 with the karyotype formula 2n = 60M+12SM+14T and ST (Gaofeng 1997). This shows the existence of variation in chromosome number of different species of the same genus with different compositions of the chromosomes. Great variations in chromosome number even among related species of decapods have been found. Moroever, No distinguishable sex chromosomes have been found in most decapods studied (Vishnoi 1972; Mittal and Dhall, 1971).

For characterization of the karyotype of a species, various bands are used as effective markers. This also facilitates characterization of hybrids due to presence of distinct chromatin block in chromosomes. These cytogenetic markers can effectively be used for

assessment of introgression in a species or population. There is no report on the localization of cytogenetic bands on *M. villosimanus* so far. Even no earlier reports on banding of any of the species of *Macrobrachium* are available. Positive C-bands identify region of heterochromatin which consist of repetitive DNA sequence and transcriptionally inactive genes (Sumner, 1998). Unlike warm-blooded animals, cold-blooded vertebrates including fish either lack or have little compartmentalization of their genomes by base composition (Medrano *et al*., 1988). NOR bands contain the genes for ribosomal RNA. The dark bands contain the facultative heterochromatic region called as G-Bands which can also be called as system of dark and light bands through the length of the euchromatin part of the chromosome. Our report of banding shows only generative banding and hence standard karyotype has not been prepared.

Decapods possess the highest chromosome number in the animal kingdom (Orian and Callan, 1957). The karyotyping procedure revealed the number and distribution of the chromosomes and the banding techniques helped to study the chromosomes structurally. *M. villosimanus* is a new species and hence need to be studied further so as to know its value and significance in the aquaculture industry. Further research on the species needs to be done on various aspects to understand the biology and genetics complements. The study on this new species of *Macrobrachium* will be helpful for maintenance and management of the species in wild stock which will in turn help in conservation of biodiversity. The present finding of our study is novel and first report of its kind. Further, bio molecular tools may be developed for characterizing the population.

CONCLUSION

Cytogenetical techniques are potential tools for genetic characterization of germplasm resources, being a basic requisite for genetic improvement program and conservation of wild gene pool. Banding techniques for chromosomes are of primary importance to cytogenetical and evolutionary studies. No earlier report of banding is available on *M. villosimanus*. Our report of banding shows only generative banding and characterization oriented banding. Detailed length/FISH oriented analysis on this species may be more useful for characterising the species.

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REFERENCES

Abe, S. and J. Muramoto, 1974. Differential staining of chromosomes of two salmonoid species, *Salvelinus leucomaenis* (Pallas) and *Salvelinus malma* (Walbaum). Proc. Jpn. Acad., 50: 507-511.

Bate, C.S., 1968. On a new genus with four new species of freshwater prawn. Proc. Zoologl. Soci. London, 1868: 363-368.

Chow, S., W.J. Dougherty and A.S. Paul, 1990. Meiotic chromosomes complements and nuclear DNA contents of four species of shrimps of the genus penaeus. J. Crustacean Biol., 10: 29-36.

Damrongphol, P., N. Eangchuan, S. Ajpru, B. Poolsanguan and B. Withyachumnarnkul, 1991. Karyotype of the giant freshwater prawn, *Macrobrachium rosenbergii*. J. Sci. Soc., 17: 57-69.

Gaofeng, Q., D. Nanshan and L. Wei, 1994. Chromosomal and karyological studies on the freshwater prawn *Macrobrachium nipponense* (Crustacea, Decapoda). Oceanologia et Limnologia Sinica, 25: 493-498.

Gaofeng, Q., 1997. Studies on chromosomes of Macrobrachium superbum heller. J. Fish. Sci. China, http://en.cnki.com.cn/Article_en/CJFDTOTAL-ZSCK701.000.htm

Holthius, L.B., 1980. Nomenclature and taxonomy, FAO species catalogue. Shrimps and Prawns of the World (an Annotated Catalogue of Species of Interest to Fisheries). FAO Fisheries Synopses No. 125 Vol. 1. Rome.

Howell, W.M. and D.A. Black, 1980. Controlled silver-staining of nucleolus rganizer regions with a protective colloidal developer: A 1-step method. Experientia, 36: 1014-1015.

Indy, J.R., L. Arias-Rodriguez, S. Paramo-Delgadillo, J. Hernandez-Guzman, A.L. d'artola Barcelo and W. Conteras-Sanchez, 2009. Cytogenetic studies of invertebrates species from Tabasco, Mexico. Proceedings of the World Aquaculture Meeting, Veracruz, Mexico, September 25-September 29, 2009, UJAT, Mexico.

Justo, C.C., M. Murofushi, K. Aida and I. Hanyu, 1991. Karyological studies on the freshwater prawn, *Macrobrachium rosenbergii*. Aquaculture, 97: 327-334.

Levan, A., K. Fredga and A.A. Sandberg, 1964. A Nomenclature for centromeric position of chromosomes. Heredites, 52: 201-220.

Medrano, L., G. Bernardi, J. Couturier, B. Dutrillaux and G. Bernardi *et al*., 1988. Chromosome banding and genome compartmentalization in fishes. Chromosoma, 96: 178-183.

Mittal, O.P. and U. Dhall, 1971. Chromosome studies in three species of freshwater decapods (Crustacea). Cytologia, 36: 633-638.

Murofushi, M., Y. Deguchi and T.H. Yoshida, 1984. Karyological study of the red swamp crayfish and the Japanese lobster by air drying method. Proc. Japan Acad. Sci. B, 5: 22-25.

Murofushi, M. and Y. Deguchi, 1990. Karyotype evolution in decapoda crustacean. Proceedings of the 2nd Asian Fisheries Forum, April 17-22, 1990, Tokyo, Japan, pp: 549-553.

Murofushi, M., T. Nakatsubo and Y. Deguchi, 1991. Karyotype Analysis of the Red Swamp Crayfish, Procambarus Clarkii by Cell Culture. In: Proceedings of the 1990 International Crustacean Conference, 2-6 July 1990: Memoirs of the Queensland Museum, Davie, P.J.F. and R.H. Quinn (Eds.). Vol. 31, Queensland Museum, Queensland, pp: 98.

Orian, A.J.E. and H.G. Callan, 1957. Chromosome numbers of gammarids. J. Marine Biololgy Assoc., 36: 129-142.

Rishi, K.K., 1989. Current Status of Fish Cytogenetics. In: Fish Genetics in India, Das, P. and A.G. Jhingaran, (Eds.). Today and Tomorrow Publishers, New Delhi, pp: 1-20.

Sharma, G.P., R. Prasad and R.P. Nayyar, 1960. Chromosome number and meiosis in three species of fishes. Res. Bull. Punjab Univ., 11: 99-103.

Sumner, A.T., 1972. A simple technique for demonstrating centromeric heterochromatin. Exp. Cell. Res., 75: 304-306.

Sumner, A.T., 1998. The mitotic chromosome. Adv. Genome Biol., 5A: 211-261.

Tan, X., J. Qin, B.N. Chen, L. Chen and X. Li, 2004. Karyological analyses on redclaw crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae). Aquaculture, 234: 65-76.

Tiwari, K.K. and L.B. Holthuis, 1996. The identity of *Macrobrachium gangeticum* (Bate), 1868 (Decapoda, Caridea, Palaemonidae). Crustaceana, 69: 922-925.

Tiwari, K.K., 1949. On a new species of palaemon from benares with a note on *Palaemon lanchesteri* (de Man). Rec. Indian Mus., 45: 333-345.

Vishnoi, D.N., 1972. Studies on the chromosomes of some Indian crustacean. Cytologia, 37: 43-51.

Xiang, J., R. Liu and L. Zhou, 1994. Chromosomes of marine shrimps with special reference to different techniques. Aquaculture, 111: 321-325.