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CONTENT

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KARYOTYPING IN THE FRESHWATER PRAWN *MACROBRACHIUM MALCOLMSONII* (MILNE-EDWARDS, 1844), DECAPODA

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ABSTRACT

Decapod karyology is one of the least studied areas. *M. malcolmsonii*, a freshwater species of the order decapoda is karyologically analysed for chromosome number, arm length, arm ratio and centromere position using a compound light microscope and an ocular micrometer. Based on the measurements using the micrometer, chromosomes were grouped. A karyogram and an ideogram were prepared. The diploid set (2n) of chromosomes from 100 metaphase spreads were found to have a modal value of 118. 26 pairs of metacentric, 9 pairs of sub-metacentric, 2 pairs of sub-metacentric/sub-telocentric, 2 pairs of subtelocentric and 20 pairs of telocentric chromosomes were observed and grouped using a standard nomenclature described by Levan *et al*., (1964).

Keywords: Aquaculture, Decapoda, *Macrobrachium malcolmsonii*, Freshwater prawn, Karyotyping, Micrometer, Diploid chromosomes.

INTRODUCTION

M. malcolmsonii a significant member of the 'species rich order -Decapoda,' is one of the largest freshwater prawns in India, of which males measures a maximum of 243 mm and females of 200 mm in size (Bhakta *et al*., 2011). Despite India, this freshwater species have wide distribution including Bangladesh and Pakistan. In southern parts of India, the species have abundant distribution in the rivers of Cauvery. Karyological studies have been carried out in its closely related species, *M. rosenbergii* (Damrongphol *et al*., 1991). Such karyological studies characterize chromosome diversity based on the morphology, size and number of chromosomes. Karyology in the order decapoda is at the least among other crustaceans orders (Torrecilla,

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2017) due to its vast number and smaller size of chromosomes. However, such karyological studies are necessary for taxonomical, breeding, chromosome manipulation and evolutionary studies in crustaceans (Mansouri *et al*., 2011).

In India, the most recent karyological study in a species of same genus was done in *M. villosimanus* resulting in a 2n value of 124 (Choudhary *et al*., 2013). However no published data on the karyological analysis of *M. malcolmsonii* is available. Therefore, this research paper outlines the methodology and outcome of karyological analysis in the freshwater decapod, *M. malcolmsonii* in some of its somatic and reproductive tissues comparatively.

MATERIALS AND METHODS

Specimen collection and transportation

Karyotype analysis was carried out using 6 captured specimens of adult male and females of *M. malcolmsonii* measuring 12.7±0.61 cm and 10.95±0.07 cm in length and 28.45±4.87 g and 16.25±0.56 g in weight respectively. *M. malcolmsonii* specimens were collected from the lower Anicut of Cauvery River at Anakarai, Tanjavur district of Tamil Nadu, India. The collected specimens were transported overnight in thick polythene bags. The polythene bags were filled with oxygen before being tightly packed.

Morphometric indices

The morphometric indices such as total length (cm), weight (g), cephalothorax length (cm), carapace length (cm), rostrum length (cm), telson length (cm), length of major chela (cm) and minor chela (cm) for male and female specimens were measured.

Maintenance of specimens

The specimens of *M. malcolmsonii* were acclimatized in cement tank of dimension 3.9x 2.3x2.2ft for a period of one week before any studies were conducted. The prawns were fed *ad libitium* with finely chopped boiled egg albumin. The water was changed every day and was kept under constant aeration. Water was maintained at a temperature of 24°C and a pH of 7 to 7.5.

Karyological analysis

In order to arrest the dividing cells at metaphase stage, the specimens were treated with colchicine. Two different methods were followed for colchicine administration.

a. Colchicine incubation – 0.3% colchicine / 6 hrs: Live adult animals were allowed to freely swim in well aerated water containing 10 ml of 0.3% colchicines for every liter of water. The prawns were incubated for 6 hours and then treated with chloroform prior to dissection.

b. Intramuscular colchicine injection – 0.5ml of 0.3% colchiccine. The adult *M. malcolmsonii* was injected intramuscularly on the ventral side of the second abdominal segment. The injected *M. malcolmsonii* was allowed to freely swim in well aerated water for 3.5 to 4 hours. It was then taken out from water and treated with chloroform prior to dissection.

Preparation of metaphase chromosome spreads

The treated specimens were dissected to procure the organs such as the gills, hepatopancreas and testis. Muscle tissues were also dissected from the site of injection of prawns that were intra-muscularly injected with colchicine. The tissues were kept for 30 minutes in 0.56% potassium chloride for hypotonic treatment. The tissues were then treated with freshly prepared Carnoy's fixative for 30 minutes. The fixed tissues were placed over a clean glass slides swabbed with ethanol and a drop of 60% acetic acid was added on top of the tissues. The tissues were chopped into very fine pieces using a blade. The macerated tissues were rinsed in a test tube with 60% acetic acid and the fine pieces were force fully pipette in and out until a clear solution was obtained. This was dropped onto slides from a height of 10 cm. The slides were allowed to air dry for an hour. A fraction of the prepared slides were also placed overnight in a laminar flow hood instead of placing in the open air to dry, in a view to obtain particulate free slides. All of these slides were then stained with freshly prepared 10% Giemsa stain solution for 30 minutes.

Determination of diploid set (2n)

Giemsa stained chromosomes of somatic tissues and testicular tissues were analysed for estimation of the diploid value 2n of *M. malcolmsonii*. Images of 100 metaphase chromosome spreads were taken at 100X magnification in a light microscope and the total number of chromosomes in each of the spreads was counted. The 2n values obtained from the spreads were tabulated. The modal value of the obtained 2n values was calculated.

Determination of arm length, arm ratio and centromere position of chromosomes

The arm length of the chromosomes was measured using an ocular micrometer (*ERMA*). The ocular micrometer was calibrated by use of a stage micrometer and the Metaphase chromosome spreads were observed and measured over the graduations of the ocular micrometer. The position of centromere was located at 400X and 1000X magnification in a light microscope. The measurement of 'p' arm, 'q' arm and the total length of chromosomes were tabulated. The metaphase chromosomes were classified based on the chromosome arm ratio as described by Levan *et al*., (1964).

Arm ratio = length of q arm/length of p arm

Chromosome grouping

The chromosomes were grouped into metacentric (group I), sub-metacentric (group II), sub-metacentric/sub-telocentric (group III), sub-telocentric (group IV), and telocentric (group V) based on their centromere position.

Karyogram

A clear metaphase chromosome spread image from hepatopancreas was used to construct a karyogram. Individual metaphase chromosomes were identified, cropped and positioned in a spread sheet in descending order of length to construct a karyogram.

Ideogram

The chromosomes were digitally replicated in Microsoft Publisher software. Each of the constructed chromosomes was arranged based on their number to construct an ideogram. A scale of 1µm was included in the ideogram.

RESULTS

The Morphometric indices of male and female M. malcolmsonii are represented in Table 1. The 2n values obtained from 100 metaphase spreads are represented in Table 2 and a modal value of 118 resulted from 40 spreads. Table 3 shows arm length, arm ratio and centromere position in 59 pairs of chromosomes. Table 4 represents chromosome groups in *M.malcolmsonii.* The Metaphase chromosome spread from hepatopancreas, testis, gills and muscles of *M.malcolmsonii* are represented in Fig. 1, 2, 3 and 4 respectively.

Table 1: Morphometric indices in male and female *M. malcolmsonii*.

Sex	TL	WT	CTL	CL	RL	TSL	Major Chela	Minor Chela
Male	13.01±1.1	31.07±9.8	5.94±0.5	3.66±0.4	3.69±0.3	1.57±0.1	14.34±1.7	6.43±1.5
Female	11.17±0.4	18.12±3.3	5.13±0.2	3.03±0.2	3.17±0.1	1.371:0.1	7.13±0.3	4.23±0.2

Data are mean values ± SD of 7 males and 3 females. TL-Total Length (cm), WT-Weight (g), CTL-Cephalothorax Length (cm), CL-Carapace Length (cm), RL-Rostrum Length (cm), TSL-Telson Length (cm).

Table 2: Diploid chromosomes number (2n) obtained from 100 metaphase spreads and representing modal value.

Chromosome Number (2n)	102	106	108	110	112	114	116	118	120	122	124	126	128
No. of spreads	1	3	5	6	5	8	12	40 (Model valve)	6	5	5	2	2

Table 3: Arm length, Arm ratio and centromere position in 59 pairs

Chromo some pair number	Total length (µm)	'p' arm length (µm)	'q' arm length (µm)	Arm length ratio (q/p)	Centromere position
1	1.98	0.90	1.08	1.2	Metacentric
2	1.80	0.90	0.90	1	Metacentiic
3	1.62	0.72	0.90	1.25	Metacentric
4	1.62	0.72	0.90	1.25	Metacentric
5	1.62	0.72	0.90	1.25	Metacentric
6	1.53	0.72	0.81	1.13	Metacentric
7	1.53	0.72	0.81	1.13	Metacentric
8	1.53	0.45	1.08	2.40	Sub-metacentric
9	1.44	0.72	0.72	1.00	Metacentric
10	1.44	0.54	0.90	1.67	Metacentric
11	1.44	0.45	0.99	2.20	Sub-metacentric
12	1.44	0.36	1.08	3.00	Sub-metacentric/sub-telocentric
13	1.35	0.63	0.72	1.14	Metacentric
14	1.35	0.63	0.72	1.14	Metacentric
15	1.35	0.54	0.81	1.50	Metacentric
16	1.35	0.36	0.99	2.75	Sub-metacentric
17	1.26	0.63	0.63	1.00	Metacentric
18	1.26	0.63	0.63	1.00	Metacentric
19	1.26	0.54	0.72	1.33	Metacentric
20	1.26	0.54	0.72	1.33	Metacentric
21	1.26	0.54	0.72	1.33	Metacentric
22	1.26	0.45	0.81	1.80	Sub-metacentric
23	1.26	0.36	0.90	2.50	Sub-metacentric
24	1.17	0.45	0.72	1.60	Metacentric
25	1.17	0.45	0.72	1.60	Metacentric
26	1.08	0.54	0.54	1.00	Metacentric
27	1.08	0.36	0.72	2,00	Sub-metacentric
28	1.08	0.27	0.81	3.00	Sub-metacentric/Sub-telocentric
29	1.08	-	1.08	"	Telocentric

30	1.08	-	1.08	∞	Telocentric
31	0.99	0.36	0.63	2.78	Sub-metacentric
32	0.99	0.27	0.72	2.67	Sub-metacentric
33	0.99	-	0.99	∞	Telocentric
34	0.90	0.36	0.54	1.50	Metacentric
35	0.90	0.36	0.54	1.50	Metacentric
36	0.90	0.36	0.54	1.50	Metacentric
37	0.90	0.18	0.72	4.00	Sub-telocentric
38	0.90	-	0.90	∞	Telocentric
39	0.90	-	0.90	∞	Telocentric
40	0.90	-	0.90	∞	Telocentric
41	0.90	-	0.90	∞	Telocentric
42	0.90	-	0.90	∞	Telocentric
43	0.90	-	0.90	∞	Telocentric
44	0.81	0.36	0.45	1.25	Metacentric
45	0.81	0.27	0.54	2.00	Sub-metacentric
46	0.81	0.18	0.63	3.50	Sub-telocentric
47	0.81	.-	0.81	∞	Telocentric
48	0.81	-	0.81	∞	Telocentric
49	0.72	0.36	0.36	1.00	Metacentric
50	0.72	0.27	0.45	1.67	Metacentric
51	0.72	-	0.72	∞	Telocentric
52	0.72	-	0.72	∞	Telocentric
53	0.72	-	0.72	∞	Telocentric
54	0.72	-	0.72	∞	Telocentric
55	0.72	-	0.72	∞	Telocentric
56	0.72	-	0.72	∞	Telocentric
57	0.72	-	0.72	∞	Telocentric
58	0.63	-	0.63	∞	Telocentric
59	0.54	-	0.54	∞	Telocentric

Table 4: Chromosome groups in *M.malcolmsonii.*

Centromere position of chromosome	Chromosome pair number
Metacentric	26
Sub-metacentric	9
Sub-metacentric/sub-telocentric	2
Sub-telocentric	2
Telocentric	20
Total number of pairs	59

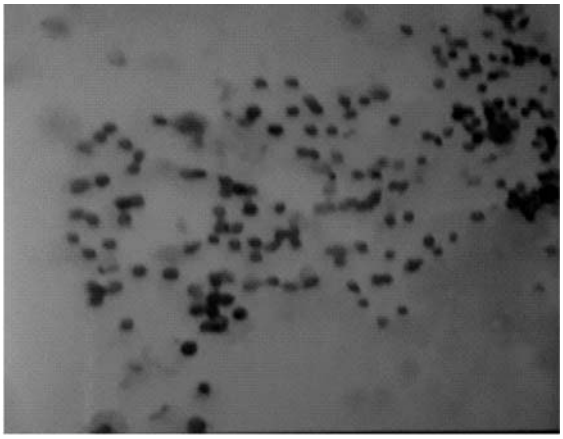

Fig. 1. Metaphase chromosome spread from hepatopancreas of *M.malcolmsonii*

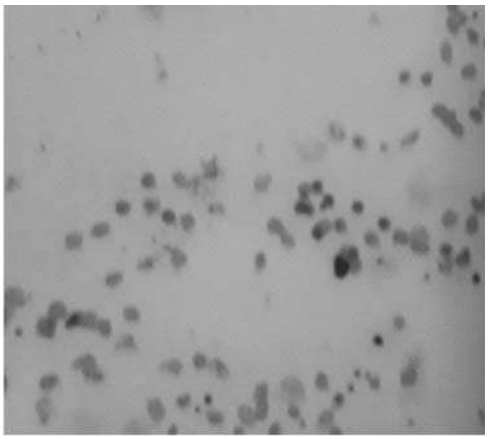

Fig. 2. Metaphase chromosome spread from testis of *M.malcolmsonii*

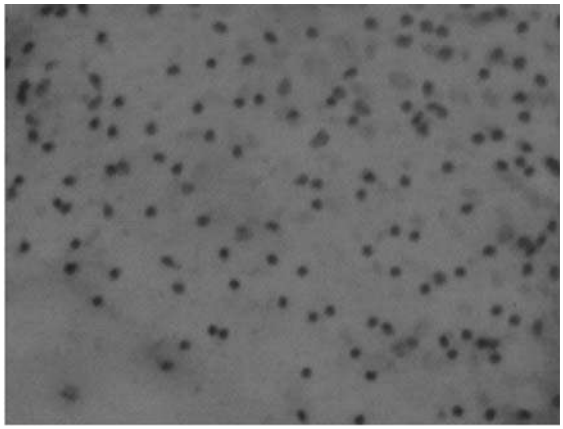

Fig. 3. Metaphase chromosome spread from gills of *M.malcolmsonii*

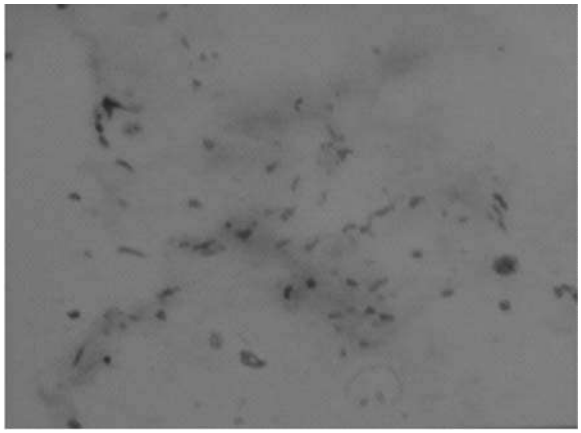

Fig. 4. Metaphase chromosome spread from muscles of *M.malcolmsonii*

DISCUSSION

In the present study, karyotypic analysis was carried out in *M. malcolmsonii*, whose the result is in parallel with the findings of Krishna *et al*., (1991), in its closest species *M. rosenbergii*. The obtained 2n value is based on the modal value from 100 metaphase chromosome spreads. Modal value is 118. However, in the present research no sex chromosomes can be distinguished. (Vishnoi, 1972) also encountered a similar status in disability to distinguish sex chromosomes in crustaceans. However, the presence of sex linked genes in *M. rosenbergii* has been reported in this species.

The variation in chromosome number analyzed can be asserted to the loss of chromosome and

to chromosome overlapping caused during the preparation of metaphase chromosome spreads. The occurrence of such errors is due to the small size of chromosomes and their closely clustered nature (Chow *et al*., 1990).

Metaphase chromosomes were visible in spreads obtained from gills, hepatopancreas, muscle and testicular tissues. The concentration and incubation of colchicine varies with tisses (Jixun *et al*., 1989).

Chromosome spreads obtained from hepatopancreatic tissue were very well defined and were used for karyotype analysis. This observation however comes in contrast with various other studies where the hepatopancreatic tissue was considered to be unsuitable for karyological studies. The inability to use hepatopancreatic tissue was traced back to its biochemical content comprising of high levels of fat, which hindered with the activity of the reagents used (Mansouri *et al*., 2011), who used only methanol:acetic acid for its fixation, without chloroform. Chloroform is known to separate lipid components from tissues (Entenman, 1957). However in the present study, the use of Carnoy's fluid containing chloroform helped to remove fat from the hepatopancreatic tissue during the fixation process and thus allowed the use of hepatopancreatic tissues for karyological studies. The chromosomes observed from metaphase chromosome spreads of hepatopancreas were properly condensed and the centromeres were visible clearly.

Chromosome spreads from gill tissues are highly prioritized in karyological studies, as gills are tissues having a single layer arrangement of cells, easily provides a large number of chromosome spreads. However, the present study does not engage for the use of gill tissues due to huge variation in the chromosome number within an individual specimen itself. Compared to other tissues used in the study, gills are the subjects of harsh environmental conditions in their natural habitats and this made the gill tissues less responsive to the hypotonic treatment.

Testicular tissues are the far preferred tissues for cytogenetic analysis Tan *et al*., (2004), providing both mitotic and meiotic spreads. Damrongphol *et al*., 1991 obtained satisfactory results with testicular tissues in *M. rosenbergii* with higher incubation period of 24 hours in 0.3% colchicines. However in the present study, testicular tissues failed to yield better results as compared to the somatic tissues. This is due to the less condensed chromosomes in the testicular tissues.

In this study, hepatopancreatic tissue showed greater levels of chromosome condensation at low colchicine incubation periods. This attributes as an advantage to the use of somatic tissues for karyological studies. The amount of somatic tissues present in the specimen far outweigh the amount of germinal tissues present in the same specimen, thus allowing the preparation of a high number of metaphase chromosome spreads from somatic tissues, irrespective of their sexes.

Interestingly, karyoptyping in crustaceans is also reported from regenerated blastemma tissues (Radwan *et al*.,2014) and antennal gland (Justo *et al*., 1991) from post larval specimens (Lakra

and Kumar, 1995) and from eggs (Ramos, 1997).

Allowing the prepared slides to stay overnight in the laminar flow hood followed by staining with Giemsa, reduced slide contamination due to settling particles, in contrast to the regular open air drying method.

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REFERENCES

Bhakta, D., Srikrishnadhas, B., Behera, S., Kumar, S., (2011). "Effect of Human Chorionic Gonadotropin on the Growth of Indian River Prawn, *Macrobrachium malcolmsonii* (H. Milne Edwards)." *J. Exp. Zool. India*.14 (1):33-36.

Choudhary, N., Sharma, R., Asthana, S., Vyas, P., Rather, M. A., Reddy, A.K., Krishna, G., (2013). "Development of Karyotype and Localization of Cytogenetic Markers in Dimua River Prawn, *Macrobrachium villosimanus* (Tiwari, 1949)." *Journal of Biological Sciences*. 13: 507-513.

Chow, S., Dougherty, W. J., Sandifer, P. A., (1990). "Meiotic Chromosome Complements and Nuclear DNA Contents of four species of Shrimps of the Genus *Penaeus*." *Journal of Crustacean Biology*. 10: 29-36.

Damrongphol, P., Eangchuan, N., Ajpru, S., Poolsanguan, B., Withyachumnarkul, B., (1991). "Karyotype of Giant Freshwater Prawn, *Macrobrachium rosenbergii*." *J. Sci. Soc.* 1(17): 57-69.

Entenman, C., (1957). "General procedures for separating lipid components of tissue." *Methods in Enzymology*. 3: 299-317.

Jixun, D., Quanqi, Z., Zhenmin, B., (1989). "Karyotype Studies on *Penaeus orientalis*." *Journal of Ocean*. 19: 97-103.

Krishna, G., Fotedar, S., Jain, A. K., (1991). "Karyotypic Studies ·of Freshwater Prawn *Machrobrachium rosenbergii*." *Journal of the Indian Fisheries Association*. 21:47-48.

Lakra, W. S., Kumar, P., (1995). "Studies on the chromosomes of two freshwater prawns, *Macrobrachium idella* and *M. scabriculum* (Crustacea, Decopoda, Palaemonidae)." *Cytobios*. 84: 147-156.

Levan, A., Fredga, K., Sandberg, A. A., (1964). "Nomenclature for Centromeric Position on Chromosomes." *Hereditas*. 52: 201-220.

Mansouri, S. M., Farahmand, H., Khalilabadi, F., (2011). "Chromosome Studies on the Marine Shrimp *Penaeus (Fenneropenaeus) merguiensis* from the Persian Gulf." *Iranian Journal of Fisheries Sciences*. 10(4): 753-760.

Radwan, H. A., Hassan, A. M., El-Azeim, S. H. A., Abbass, A. A., (2014). "Cytogenetic Characterization and Genetic Variations between Freshwater Crayfish *Procambarus clarkia* in Egypt." *Research Journal of Pharmaceutical, Biological and chemical sciences*. 5(2): 1801- 1816.

Ramos, R. C., (1997). "Chromosome Studies on the Marine Shrimp *Penaeus vannamei* and *P. calforniensis* (Decapoda)." *Journal of Crustacean Biology*. 17(4): 666-673.

Tan, X., Jian, G, Q., Chen, B., Chene, L., Li, X., (2004). "Karyological Analyses on Redclaw crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae)." *Aquaculture*. 234:65-76.

Torrecilla, Z., Lage, A.M., Perina A., Ortegon, E.G., Tizon, G.A.M., (2017). "Comparative cytogenetic analysis of marine *Palaemon* species reveals a X1XIX2X2/XIX2Y sex chromosome system in *Palaemon elegans*." *Front. Zool*. 14: 47.

Vishnoi, D. N., (1972). "Studies on the Chromosomes of some Indian Crustaceans". *Cytologia*. 37(1): 43-51.

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