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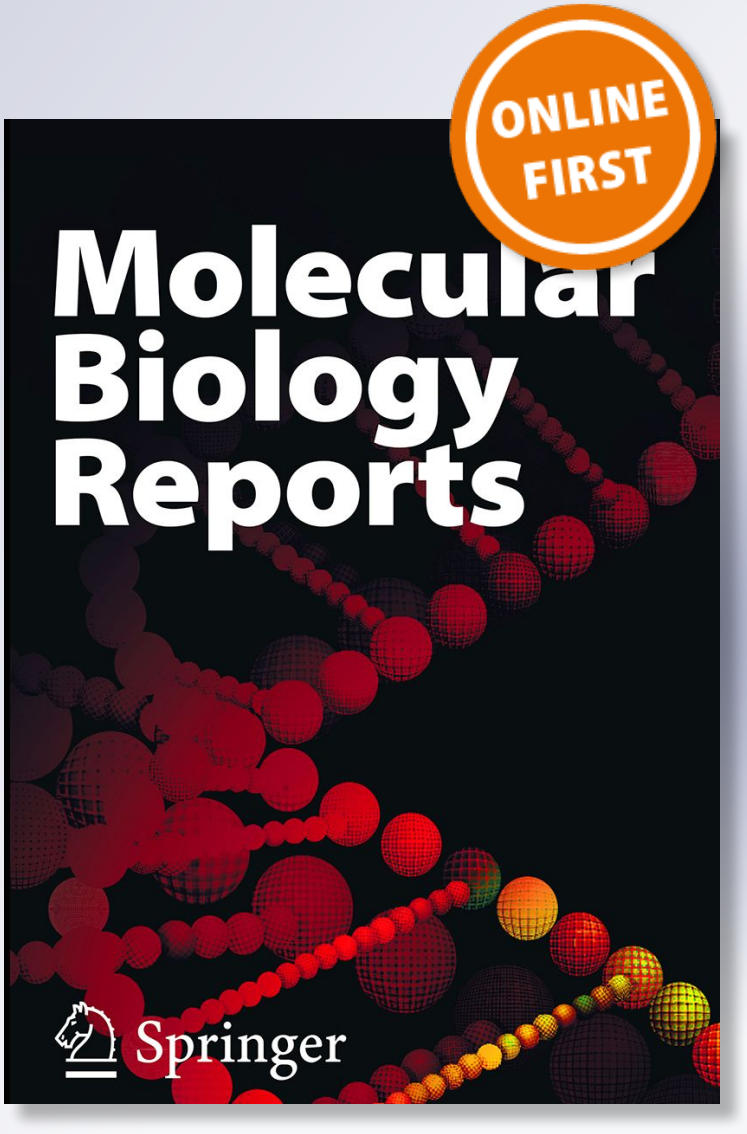

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ORIGINAL ARTICLE

Genome size estimation of brackishwater fishes and penaeid shrimps by flow cytometry

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Abstract
Flow cytometry was used for estimating the genome size of five brackishwater finfish and four shrimp species. The genome size for *Lutjanus argentimaculatus* was 0.95 ± 0.10 and 0.79 ± 0.01 pg for *Scatophagus argus*. The genome sizes for *Chanos chanos* (0.72 ± 0.01 pg), *Etroplus suratensis* (1.71 ± 0.16 pg) and *Liza macrolepis* (0.87 ± 0.02 pg) which are important aquaculture species are reported for the first time in this study. The phylogenetic tree constructed using sixty-seven sequence accessions of cytochrome c oxidase subunit 1 (COI) gene of *Lates calcarifer* revealed two separate clades. The Indian *Lates calcarifer* species with estimated genome size of 0.44 ± 0.02 pg belonged to a clade different than that of South East Asia and Australia reported to have larger genome size. The genome size for the four major species of genus *Penaeus* (*Penaeus monodon, Penaeus indicus, Penaeus vannamei and Penaeus japonicus*) were found in similar range. The genome size of female shrimps ranged from 2.91 ± 0.03 pg (*P. monodon*) to 2.14 ± 0.02 pg (*P. japonicus*). In male shrimps, the genome size ranged from 2.86 ± 0.06 pg (*P. monodon*) to 2.19 ± 0.02 pg (*P. indicus*). Significant difference was observed in the genome size between male and female shrimp of all species except in *P. monodon*. The highest relative difference of 12.78% was observed in the genome size between the either sex in *P. indicus*. The interspecific relative difference of 30.59% in genome size was highest between the male shrimps of *P. monodon* and *P. indicus* and 35.98% between the female shrimps of *P. monodon* and *P. japonicus*. The stored gills and pleopod tissues could be successfully used up to 3 weeks to estimate the genome size in shrimps.

Keywords Genome size · Flow cytometry · Brackishwater · Finfish · Shrimp

Introduction

Various fish and shellfish species are being cultured globally, amongst them brackishwater fishes and shrimps constitutes an important food resource. The brackishwater aquaculture fish species such as large scale mullet, milk fish, Asian sea bass, pearlspot and spotted scat are important candidates for breeding and culture and have commercial value for human consumption or has a potential for ornamental trade. In India, with introduction of exotic shrimp species *Penaeus vannamei*, there is rapid growth of shrimp aquaculture industry in the recent years with cultured shrimp production estimated to be 426,500 MT. The other major shrimp species which are cultured and contribute in shrimp production are *Penaeus monodon* and *Penaeus indicus*.

With the rapid advancement in next generation sequencing (NGS) techniques, there is a concentrated research effort worldwide to decipher whole genome sequence of various aquaculture fish and shellfish species including the non-model organisms to understand the organization and evolutionary pattern of their genomes. A whole genome sequence information generated by NGS allows identification of candidate genes and molecular markers associated with desirable genetic traits. Deciphering of the whole genome will have very high impact in the near future for molecular assisted aquaculture breeding programmes [1] and in understanding

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evolution and population genomics in fishes [2]. Prior estimation of the genome size is essential in understanding the genome content and the read depth in NGS especially where no reference genome exits for a species.

Various methods have been used to estimate the genome size in fish and shell fish species including the use of real-time quantitative PCR in estimating genome size of southern platyfish, *Xiphophorus maculatus* [3]. The other reliable and faster technology of estimation of genome size is based on flow cytometry analysis in which the individual cell's DNA is stained and analyzed by fluorescence measurement using propidium iodide [4] or DAPI [5]. Another technique of Feulgen image analysis densitometry (FIAD) using monolayer of nucleated cells, is also reported to be widely used for estimation of genome size in crustaceans [6]. The flow cytometry technique is preferred over other methodologies for accurate estimation of the genome size [7].

Numerous reports are available in correlating the genome size with phenotype, ecological habitat and phylogenetic trends in several organisms [8]. Correlation of the genome size with other physiological and morphological characteristics of fish requires in-depth studies and remains to be established. For example, positive correlation of genome size on longevity shown in Actinoptergyian fishes [9], is debatable and not conclusive based on the arguments and observations of Gregory [10]. The other studies have used genome size estimation to correlate with physiological, cytological and metabolic traits. Strong correlation has been reported between the cell and nuclear size with genome size in ray-finned and cartilaginous fishes [11]. The genome-size estimates carried out for more than 500 ray-finned and cartilaginous fishes based on Feulgen staining indicated existence of large diversity in the genome size and no correlation was found between genome size and metabolic rates in ray-finned fishes, however positive correlation was observed with egg size of fishes [12]. Determination of genome size in eight commercially important fish species in China by flow cytometry revealed that the more evolved fishes had smaller genome size as compared to the less evolved ones and the genome size positively correlated with size of fish erythrocyte nucleus size and chromosome number using propidium iodide based flow cytometry [5].

In case of crustaceans, studies have been documented on genome size estimation and its correlation with other parameters. A large interspecific and intraspecific variation in genome size was observed to exist in 39 species of genus *Synalpheus* (snapping shrimp) by FIAD analysis and no correlation could be found with the body size. In four *Synalpheus* species analysed, the genome size positively related to chromosome size and not number [13]. Based on the taxonomy dendrogram constructed from genome size database, a recent study has shown that the genome size was weakly related to phylogeny in crustaceans, whereas in insects it was phylogeny dependent [14]. Sequence assembly of shrimp whole genomes in particular, is challenging and difficult because of complex nature and large size of the genome. Interestingly, arctic marine shrimps such as polar shrimp *Sclerocrangon ferox* is reported to have very large genomes size of 40.0 pg [15].

In this study, propidium iodide staining with flow cytometry was used to estimate the genome size of six brackishwater finfish and four shrimp species as an approach to understand the genome composition in these species which would aid in genome based breeding programmes in future. The whole genome sequence remains to be deciphered in these species except for Asian sea bass of South East Asian population (Singapore) in which ~ 670 Mb genome assembly has been reported [16].

Materials and methods

Fish and shrimp samples

In this study we used a total of six fish species. The fish species of either sex used for genome size estimation were red snapper (*Lutjanus argentimaculatus*) (n = 5), Asian sea bass (*Lates calcarifer*) (n = 5), milkfish (*Chanos chanos*) (n = 5), pearl spot (*Etroplus suratensis*) (n = 5), spotted scat (*Scatophagus argus*) (n = 5) and large scale mullet (*Liza macrolepis*) (n = 5). The four important species of genus *Penaeus* included *Penaeus monodon* (n = 6), *Penaeus japonicus* (n = 6), *Penaeus vannamei* (n = 6) and *Penaeus indicus* (n = 6). The replicates included three each of males and female shrimps. The blood samples and length and weight measurements were taken from the red snapper, Asian sea bass, milkfish and spotted scat which were maintained in the brood stock facility of the fish hatchery of the Institute located at East coast of India, Chennai. The samples of pearl spot, large scale mullet and shrimp species were collected from the wild. The gender of the fishes could not be determined as the fishes were maintained as broodstock and the wild samples were in immature stage. The species identification of the samples collected from wild was carried out using primers for mitochondrial cytochrome c oxidase subunit I gene (COI) for shrimp [17] and fish [18].

Fish blood cells

During the blood collection, the fishes were anesthetized with 2-phenoxyethanol (Sigma-Aldrich, Co., St. Louis, MO, USA). The blood was collected using 5-ml syringe containing 0.01 M phosphate-buffered saline (PBS) from the caudal vein of the fish. The fish blood was prepared for propidium iodide (PI) staining following the procedure as described below for shrimp haemocytes.

Shrimp haemocyte collection and preparation

The haemolymph was drawn using 1-ml syringe prefilled with 0.01 M of PBS from the first abdominal segment in the ventral sinus of the shrimp. Shrimp haemolymph was centrifuged at 5000 rpm for 8 min to sediment the haemocytes. Washing of cells was carried out with 0.01 M PBS followed by fixing of cells with ice cold 70% ethanol for 2 h at 4 °C. The cells were washed twice with 0.01 M PBS before proceeding with the propidium iodide (PI) staining as described by Zhu et al. [5] with minor modifications. To the cell pellet 1 ml of propidium iodide (PI) (Sigma-Aldrich, Co., St. Louis, MO, USA) staining solution (1% NP40 (Sigma-Aldrich, Co., St. Louis, MO, USA), 100 µg/mL DNase-free RNase A (Qiagen, Germany), 10 µg/mL PI dye in PBS) was added followed by incubation in dark for 30 min at room temperature. The sample was filtered through sterile cell strainer of size 40 µm (Corning, Sigma-Aldrich, Co., St. Louis, MO, USA) and analysed in flow cytometer. Similar procedure was carried out to process chicken red blood cells (RBCs) which was used as standard sample. The PI stained shrimp and chicken samples were mixed in the ratio of 1:1 volume to detect the comparative result of unknown and standard samples in flow cytometer.

Flow cytometry analysis

The genome size estimation was performed using BD Accuri™ C6 plus flow cytometer (BD Biosciences, USA). The flow cytometer was calibrated for its specificity using BD™ cytometer setup with tracking beads. For setup and verification of the doublet discrimination of flow cytometer BD™ DNA QC particles were used. Gating was done to select unified singlet population of cells (2C). Genome size was estimated with the corresponding mean value of the unknown and with the known genome size of chicken (*Gallus domesticus*) 1.25 pg [19]. Flow cytometry analysis was carried out using laser excitation at 488 nm, and with minimum 10,000 events (cells) per sample. The coefficient of variation (CV) and mean fluorescence intensity of diploid G0/G1 nuclei were obtained using BD Accuri C6 plus software. The mean CVs were less than $8 \pm 0.5\%$ for both sample and standard. The DNA content size was estimated using the standard formula for genome size (in pg) = (Sample fluorescence channel number FL/Chicken fluorescence channel number FL) × 1.25 pg. The genome size was calculated and expressed as haploid DNA nuclear content 1 pg = 978 Mbp [20]. Statistical significance was estimated using one way ANOVA by SPSS software.

Genome size estimation in stored shrimp samples

An experiment was conducted to determine the variability of estimated genome size in different shrimp tissues such as haemolymph, gills and pleopod and at different intervals of short term storage at − 80 °C. *P. vannmaei* was taken as test sample and analysed at 1 day, 1, 2 and 3 weeks time intervals. The genome size estimation by flow cytometry was carried out in haemolymph, gills and pleopods in three replicate shrimp samples following the procedure reported earlier using LB01 buffer for shrimp tissues [13]. Briefly, gills and pleopod were collected on the first day of the experiment from 12 shrimps and stored directly in − 80 °C. The haemolymph was collected from these 12 shrimps in an equal volume of phosphate buffer using sterile 2 ml syringes and were stored at 4 °C for further analysis. Tissues and haemolymph collected from three shrimp were processed at each of the storage time point and used for estimation of genome size. The stored haemolymph and tissues (approx. 150 mg tissues) were processed at different time intervals by homogenizing in 500 µL of ice-cold lysis LB01 buffer (15 mM Tris, 2 mM Na_2EDTA, 0.5 mM spermine.4HCl, 80 mM KCl, 20 mM NaCl, 15 mM β-mercaptoethanol, 0.1% (v/v) Triton X-100, pH 7.5). The homozenized gills and pleopod samples were then filtered using sterile cell strainers of 40 µm size (Corning, Sigma-Aldrich, Co., St. Louis, MO, USA) by centrifugation. The filtrate was collected and stained using 2 µL (6 µg/mL) of RNase and 12 µl (24 µg/mL) propidium iodide solution per sample. In the case of haemolymph, the cell pellet was washed twice using LB01 buffer and processed for staining. The stained samples were kept in dark for 30 min and analysed in flow cytometry as described above with chicken erythrocytes used as the primary standard.

Phylogenetic analysis

Sixty-seven GenBank sequence accessions of cytochrome c oxidase subunit 1 (COI) gene belonging to *L. calcarifer* specimens belonging to India, Bangladesh, Myanmar, Indonesia, Singapore, Thailand, Malaysia, China and Australia were used for phylogentic analysis. An accession of *L. niloticus* was taken as outgroup for building phylogenetic tree. Initially all accessions were aligned in BioEdit version 7.2.5 [21] to obtain a consensus sequence. The best fit partitioning scheme and model for consensus alignment were obtained using PartitionFinder v2.1.1 [22, 23]. Maximum Likelihood tree was constructed with best partitioning scheme and GTR + G + X model in RAxML version 8.2.9 [24].

Statistical analysis

The results obtained were analysed using SPSS software for statistical inference. Experimental values with p value < 0.05 were considered to be statistically significant. The haploid DNA contents (C-values, in picograms) available for fish and shrimp species in Animal Genome Size Database [25] was used for comparative analysis.

Results

Flow cytometer analysis

The histograms of fish species and histograms of male and females shrimp samples obtained by PI fluorescence dye excitation and counts representing the cell population are shown in Figs. 1, 2 respectively.

Genome size estimation

The average estimated genome size along with measured weight and length from five individuals each of six fish species is shown in Table 1. *Etroplus suratensis* showed the largest genome size of 1.71 ± 0.16 (pg) whereas, *L. calcarifer* showed the least genome size of 0.44 ± 0.02 (pg).

The average estimated genome size along with measured weight and length from three individuals each of either sex of four shrimp species is shown in Table 2. The genome size of female shrimps ranged from 2.14 ± 0.02 pg (*P. japonicus*) to 2.91 ± 0.03 pg (*P. monodon*) and in the male shrimps the genome size ranged from 2.19 ± 0.02 pg (*P. indicus*) to 2.86 ± 0.06 (*P. monodon*). Significant difference (p value < 0.05) was observed in the genome size between male and female shrimp of all species except in *P. monodon*. The highest relative difference of 12.78% was observed in the genome size between the either sex in *P. indicus*. Whereas, the variation (relative difference) in the genome size between the either sex in other shrimps was observed to be in small range in *P. vannamei* (5.93%), *P. japonicus* (7.47%) and *P.*

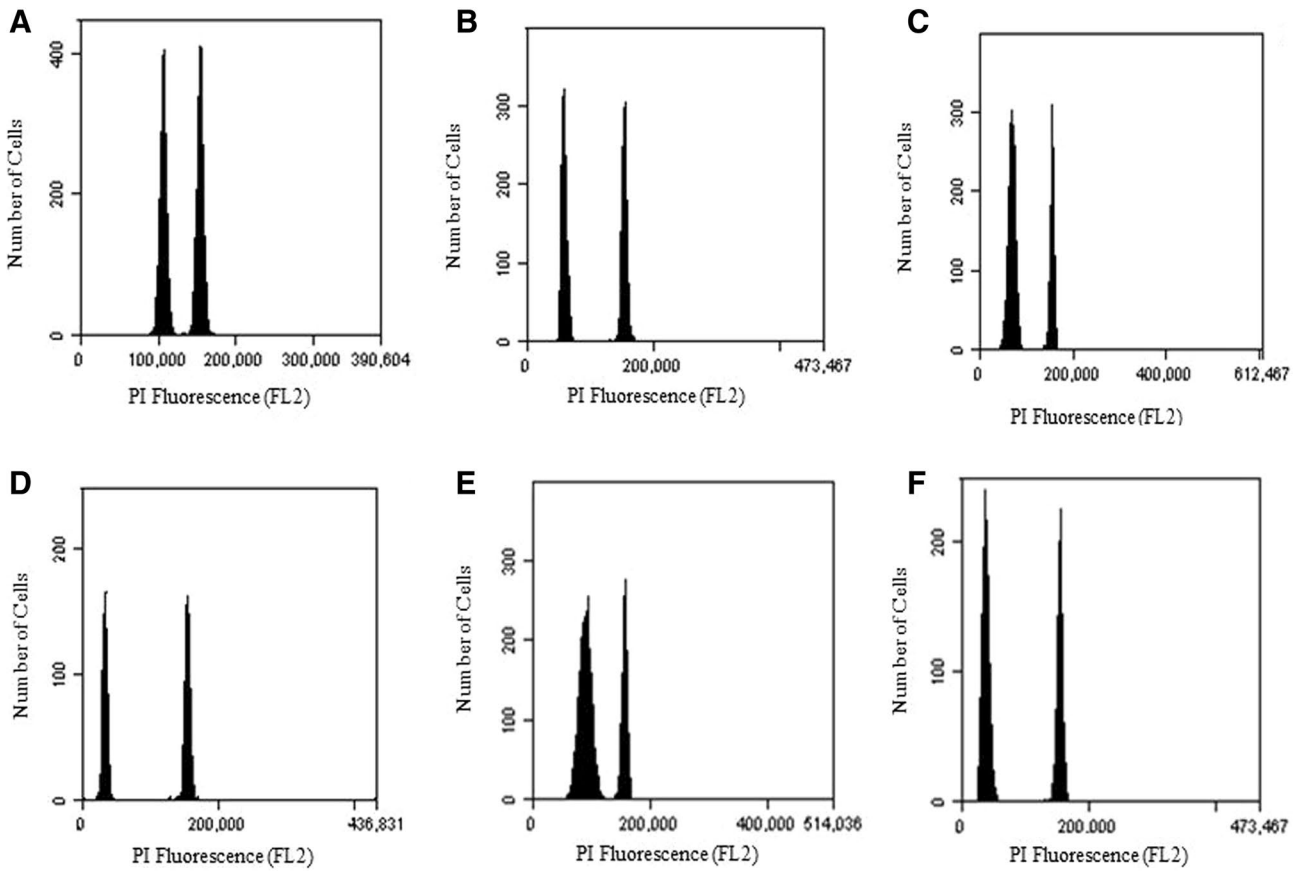

Fig. 1 Flow cytometry histograms of samples (first peak) and chicken erythrocytes (second peak) obtained by PI fluorescence dye excitation and counts representing the cell population **a** *Etroplus suratensis*, **b** *Scatophagus argus*, **c** *Chanos chanos*, **d** *Liza macrolepis*, **e** *Lutjanus argentimaculatus* and **f** *Lates calcarifer*

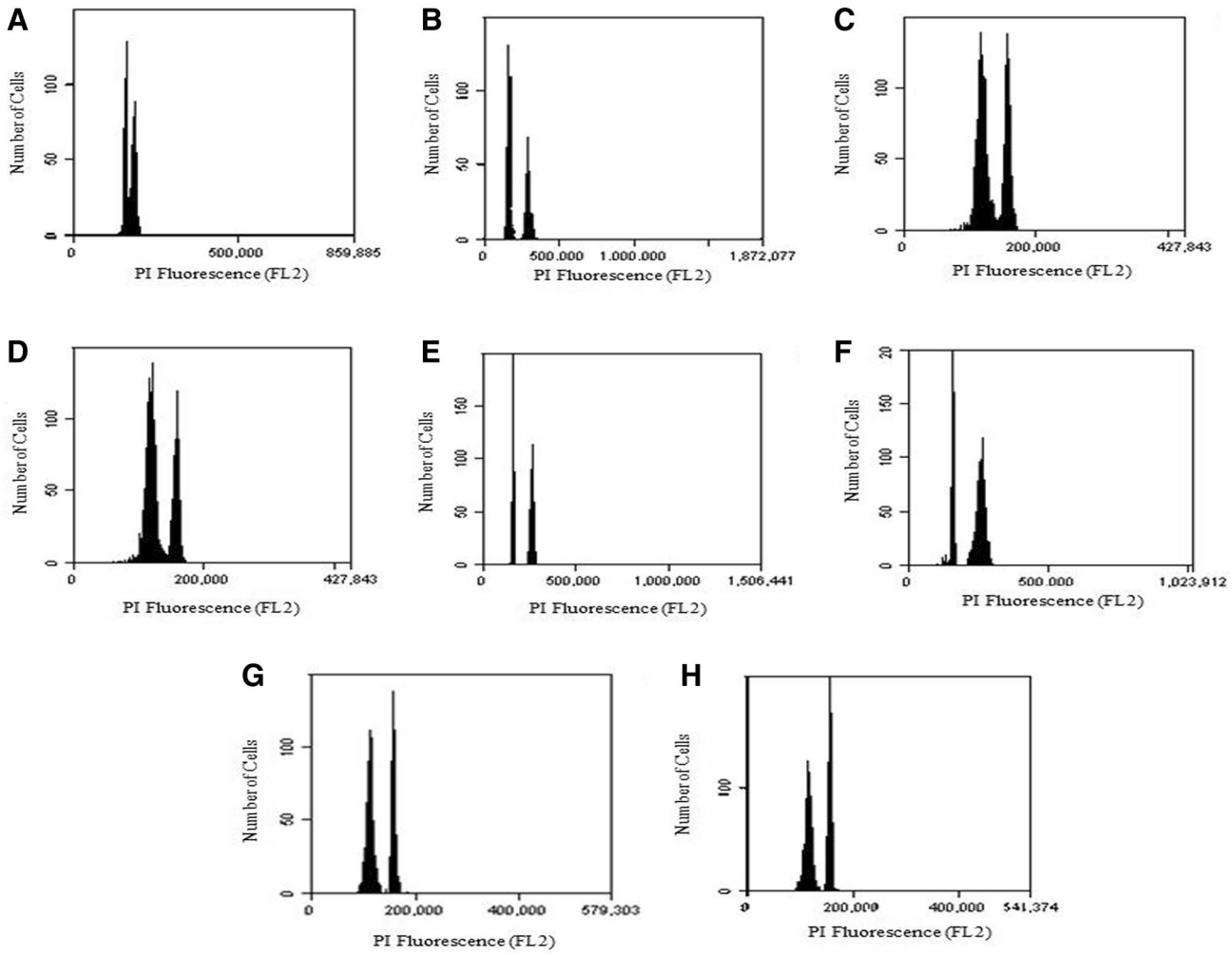

Fig. 2 Flow cytometry histograms of samples (first peak) and chicken erythrocytes (second peak) obtained by PI fluorescence dye excitation and counts representing the cell population **a** *Penaeus indicus*—female, **b** *Penaeus indicus*—male, **c** *Penaeus japonicus*—female, **d** *Penaeus japonicus*—male, **e** *Penaeus monodon*—female, **f** *Penaeus monodon*—male, **g** *Penaeus vannamei*—female and **h** *Penaeus vannamei*—male

Table 1 The estimated genome size along with measured weight and length from six fish species

Species	Weight (g)	Length (cm)	Genome size (pg)
Chanos chanos	1929.5–2070.5	98.5–99.5	0.72 ± 0.01
Lutjanus argentimaculatus	1419.2–1580.8	97.5–98.5	0.95 ± 0.10
Etroplus suratensis	97.7–98.3	14.5–19.5	1.71 ± 0.16
Scatophagus argus	149.3–150.6	22.4–25.6	0.79 ± 0.01
Liza macrolepis	98.7–97.3	20.4–21.6	0.87 ± 0.02
Lates calcarifer	2416.5–2583.5	101.1–102.1	0.44 ± 0.02

The range of weight, length and average genome size was estimated in five replicates each of fish species

monodon (1.74%). The interspecific relative difference of 30.59% in genome size was highest between the male shrimps of *P. monodon* and *P. indicus* and in the female shrimps, the highest difference of 35.98% was observed between *P. monodon* and *P. japonicus*. Significant difference was also observed in the genome size between all species of shrimp.

Genome size estimation in stored shrimp samples

Using the lysis LB01 buffer, genome size could also be estimated successfully in gills and pleopod in all replicates of shrimp samples at all storage time intervals. However, in haemolymph, at 3 weeks of storage time, the genome size

Table 2 The estimated genome size with measured weight and length from four shrimp species

Species	Weight (g)	Length (cm)	Genome size (pg)
Penaeus monodon	17.5–20.5 (M)	14.5–15.5 (M)	2.86 ± 0.06 (M)
	19.2–20.8 (F)	13.8–14.2 (F)	2.91 ± 0.03 (F)
Penaeus japonicus	19.2–20.8 (M)	13.8–16.2 (M)	2.30 ± 0.04 (M)*
	17.5–22.5 (F)	14.5–17.5 (F)	2.14 ± 0.02 (F)*
Penaeus vannamei	15.5–18.3 (M)	11.1–14.2 (M)	2.32 ± 0.13 (M)*
	14.6–19.6 (F)	12.1–15.0 (F)	2.19 ± 0.03 (F)*
Penaeus indicus	17.5–18.5 (M)	12.2–15.7 (M)	2.19 ± 0.02 (M)*
	18.8–19.2 (F)	14.6–15.5 (F)	2.47 ± 0.04 (F)*

The range of weight, length and average genome size was estimated in three replicates each of male and female shrimp species. Statistically significant (p value < 0.05) values in the genome size is indicated by *

could not be estimated due to low cell count and cell debris (Fig. 3).

Genome size in the shrimp haemolymph ranged from 2.44 ± 0.04 to 2.49 ± 0.03 pg with relative difference of 0.4% and 0.01 pg of absolute difference between 1 day versus 2 weeks storage duration.

Similar relative difference of 0.4% and 0.01 pg of absolute difference between 1 day versus 3 weeks storage duration was observed in the genome size estimated in gill tissues which ranged from 2.46 ± 0.04 to 2.52 ± 0.02 pg. The maximum relative difference of 1.11% and 0.03 pg of absolute difference between 1 day versus 2 weeks storage duration was observed in the genome size estimated in shrimp pleopod tissue which ranged from 2.62 ± 0.17 to 2.69 ± 0.06 pg (Table 3).

Phylogenetic analysis

Based on the aligned sequences, the phylogenetic analysis of 67 *L. calcarifer* COI gene revealed two distinct clades of species. Twenty-one individuals of *L. calcarifer* representing India, Myanmar and Bangladesh formed one clade. Whereas, the second clade represented the 46 samples of *L. calcarifer* from Indonesia, Singapore, Thailand, Malaysia, China and Australia (Fig. 4).

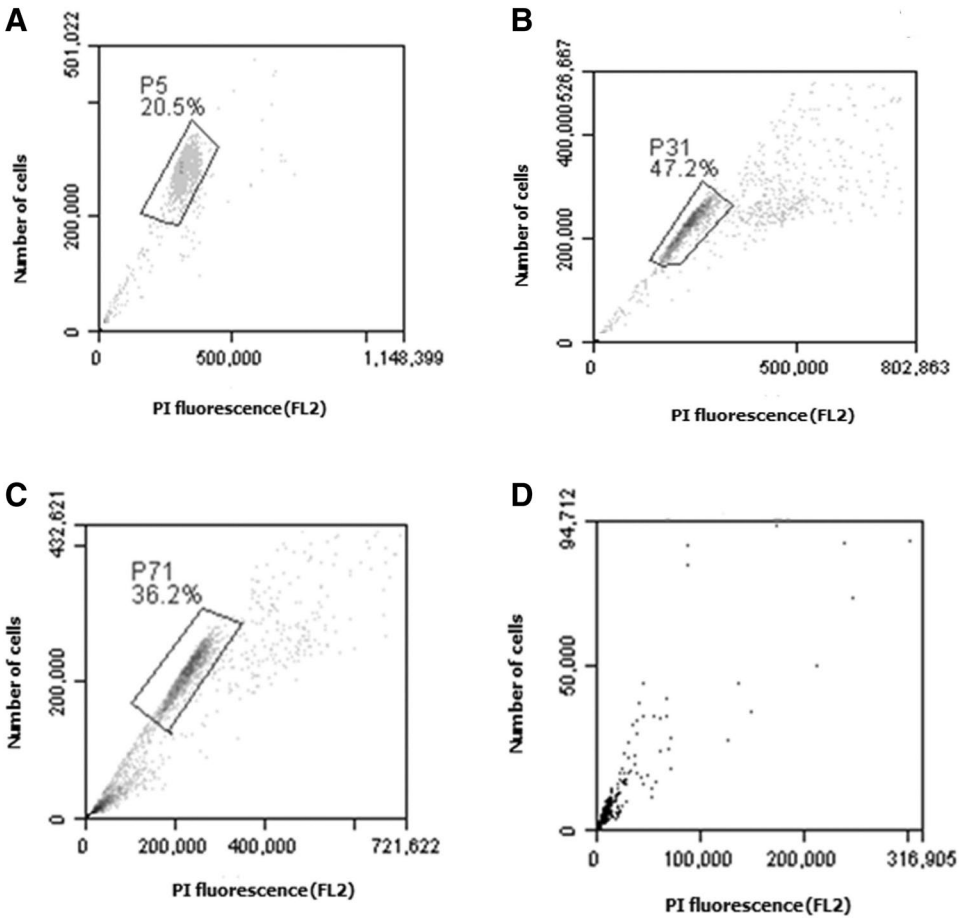

Fig. 3 Flow cytometry dot plots of haemolymph samples at different time intervals obtained by PI fluorescence. **a** 1 day, **b** 1 week, **c** 2 weeks and **d** 3 weeks

Table 3 Genome size estimation in different shrimp tissues at different storage time intervals

Storage duration of preserved sample	Sample type	1C genome size (pg)	Relative difference (%)	Absolute difference (pg)
1 day	Haemolymph	2.49 ± 0.03	0.40	0.01
1 week	Haemolymph	2.44 ± 0.04		
2 weeks	Haemolymph	2.48 ± 0.01		
3 weeks	Haemolymph	ND		
1 day	Gills	2.49 ± 0.00	0.40	0.01
1 week	Gills	2.52 ± 0.02		
2 weeks	Gills	2.46 ± 0.04		
3 weeks	Gills	2.50 ± 0.04		
1 day	Pleopod	2.69 ± 0.06	1.11	0.03
1 week	Pleopod	2.62 ± 0.17		
2 weeks	Pleopod	2.68 ± 0.02		
3 weeks	Pleopod	2.66 ± 0.21		

The relative differences and absolute differences in genome size are shown between 1 day versus 2 weeks storage duration for haemolymph samples and between 1 day versus 3 weeks storage for gills and pleopod samples

ND Not determined

Discussion

The milkfish, *Chanos chanos* native to regions in the Indian Oceans and found in tropical waters is an important aquaculture species. However, there is no information on the genome size of this fish. This is the first time report of genome size (0.72 ± 0.01 pg) for milkfish estimated by flow cytometry. It will be interesting to know the genome of this fish which is listed to be sequenced under 10 K pilot project [26]. The genome size of *Lutjanus argentimaculatus* (0.95 ± 0.10 pg) estimated in this study is marginally lesser than the genome size reported for *Lutjanus malabaricus* (0.98 pg) using FIAD [12]. *Scatophagus argus* genome size of 0.79 pg estimated in the present study, is very similar to the estimated genome size with C-value (pg) of 0.77 reported for the same species using Bulk Fluorometric Assay [27].

Lates calcarifer, is native to the Indian and Western Pacific Oceans which includes Australia, Southeast and Eastern Asia, and India. *L. calcarifer* is reported to have genome size of C-value (pg) of 0.7 pg (700 Mb) which was estimated using FIAD [12]. A recent study indicated ~ 670 Mb genome assembly of a partially inbred F2 *L. calcarifer* sample from SE Asia using long sequence reads [16]. In the present study, we obtained 0.44 ± 0.02 pg genome size for *L. calcarifer*, from India, which is less than the reported genome size for this fish [12, 16]. No previous data is available on the genome size estimate of *L. calcarifer* from Indian subcontinent. The difference in genome size of *L. calcarifer* from India (0.44 Mb) estimated in this study with that of other reports of genome size of *L. calcarifer* collected from Sydney fish markets (700 Mb) [12] and from SE Asia (~ 670 Mb) [16], can be due to genetic difference which exists in species belonging to different geographical locations. *L. calcarifer* from India in phylogenetic analysis of COI gene represented in one clade, whereas the larger genome size of *L. calcarifer* from SE Asia and Australia clearly belonged to a distinct second clade (Fig. 4). Earlier reports based on 14 polymorphic microsatellites, the phylogenetic analysis of the wild populations of *L. calcarifer* from Singapore, Australia, Malaysia, Thailand, Indonesia and Taiwan revealed that Australian stock was genetically different from Southeast Asian and Taiwanese stocks [28]. Similar observations in existence of two distinct species has also been reported between the Indian subcontinent plus Myanmar stocks and Southeast Asia plus Northern Australia stocks based on mitochondrial genes sequence variations [29] and between Australian stocks and Myanmar stocks [30].

The genome size of *Etroplus suratensis* and *Liza macrolepis* has not been reported by others so far. *Liza macrolepis* with 0.87 pg of genome size was higher as compared to the genome size reported for another closely related species *Liza ramada* (0.79 pg) [31].

Using the database available for genome size of fishes [25], no correlation could be inferred between the genome size of the fish to the taxonomic order the fish belonged. *Chanos chanos* belonging to taxonomic order Gonorynchiformes clustered with *Zebrasoma scopas* and *Channa punctata* of order Perciformes having same genome size of 0.72 pg. *Scatophagus argus* and *Acanthurus triostegus* of taxonomic order Perciformes clustered with *Liza ramada* of Mugiliformes taxonomic order having same genome size of 0.79 pg. *Lutjanus argentimaculatus* and *Oreochromis spilurus spilurus* belonging to two different taxonomic family of

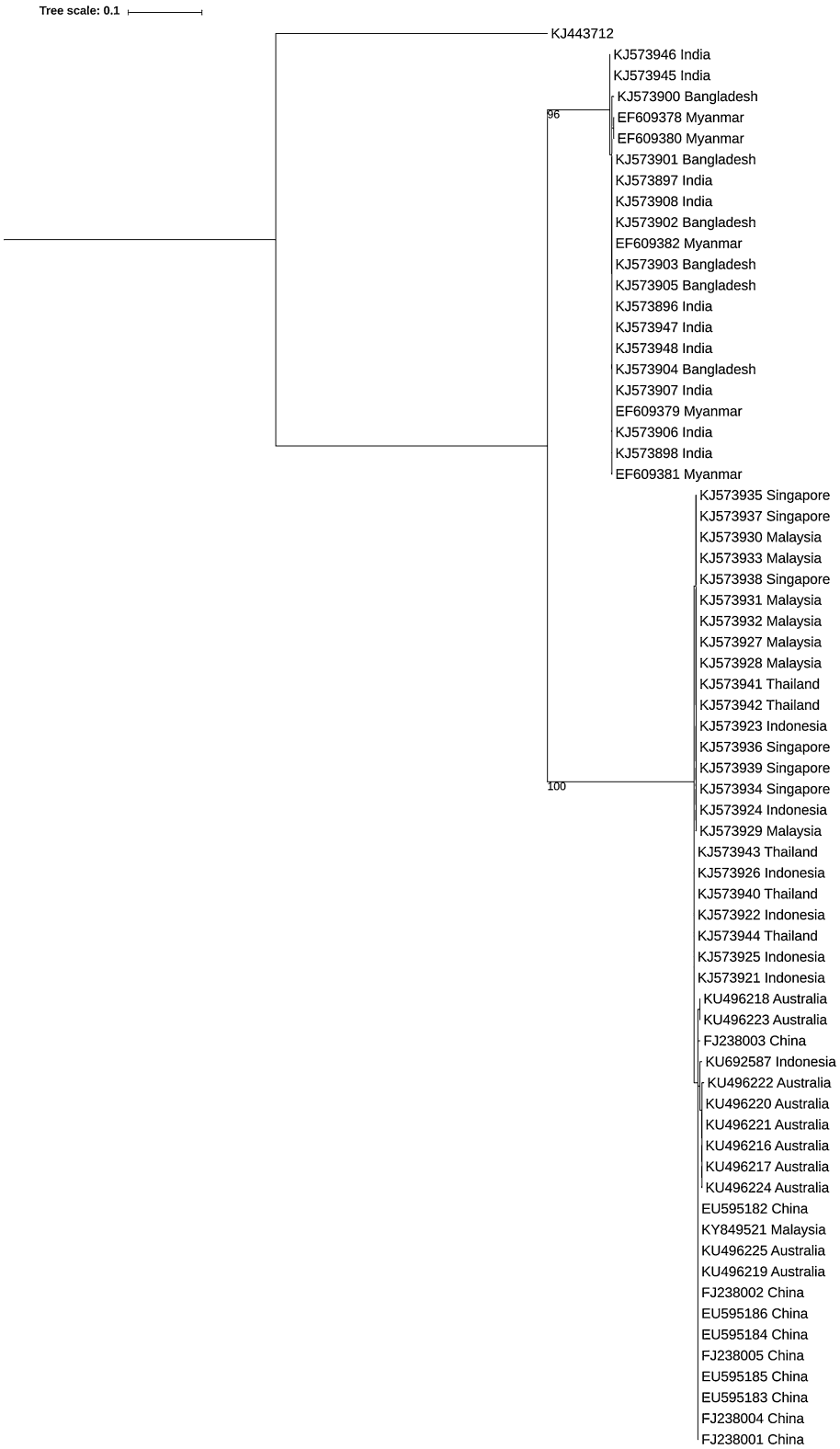

Fig. 4 The phylogenetic analysis of 67 *Lates calcarifer* COI gene revealing two distinct clades of the species

Lutjanidae and Cichlidae respectively had similar genome size of 0.95 pg.

The shrimp genome size with C-value (pg) 2.53 has been estimated for *P. monodon* using shrimp haemocytes in flow cytometry [32]. In present study, the genome size of female *P. monodon* (2.91 ± 0.03 pg) was not significantly different than that of the male shrimp 2.86 ± 0.06.

The genome size estimated in *P. japonicus* for males (2.3 ± 0.04 pg) and in females (2.14 ± 0.02 pg) is less and vary with the observations reported for genome size estimated by others in gill tissues of *P. japonicus* with C-value of 2.83 pg [33]. The difference in the cell type analysed in our study (haemocytes) and gill tissues used by others may be the reason for difference in genome size estimation. The tissue sampling, tissue storage duration, fixatives and standardization of protocols is critical for accuracy of results by flow cytometry [34]. Variability in data is reported to occur using different fluorescent dyes in flow cytometry [5] and tissue storage conditions using FIAD and flow cytometry [6].

The genome size estimated for *P. vannamei* in the present study for males (2.32 ± 0.13 pg) and in females (2.19 ± 0.03 pg) is less than the genome size reported for *P. vannamei* using flow cytometry (2.89 pg) and FIAD (2.67) respectively [6]. This variation may also be due to the method of preservation, type of tissue and method used for analysis. The relative difference of 2.49% and absolute difference of 0.07 pg is reported for the *P. vannamei* genome size estimate between for flash-frozen and ethanol-preserved samples by FIAD, while a difference of 0.22 pg difference in shrimp genome size estimate was observed between flow cytometry and FIAD analysis [6].

To date, no flow cytometry-based estimates of the *P. indicus* genome size has been published. However, our results of genome size for males (2.19 ± 0.02 pg) and females (2.47 ± 0.04 pg) were in the same range of genome as estimated for the other three species of shrimp in this study.

Our results showing significant difference in the genome size between male and female shrimp of all species except in *P. monodon* and significant difference in the genome size between all species of shrimp are in partial agreement with results reported by other workers. In the study conducted by Chow et al. [35], the flow cytometry analysis of DNA content of four shrimp species (*P. aztecus, P. duorarum, P. vannamei and P. setiferus*) showed no significant difference in genome size between sexes, however significant difference in genome among species was observed.

Limited information is available on use of shrimp tissue material and storage conditions for genome size estimation using flow cytometry. A study based on 37 species of crustaceans showed the use of gill tissue both in flash-frozen versus ethanol-preserved by Feulgen image analysis was desirable [6]. This study indicated that genome size can be estimated in shrimp gills and pleopod stored at − 80 °C till 3 weeks of storage time. The genome size could be estimated in haemolymph stored till 2 weeks and not at 3 weeks. The results obtained in the present study revealed very less relative difference of 0.4% and absolute difference of 0.01 pg using gill tissues stored at − 80 °C till 3 weeks of storage time. The haemolymph also showed similar range of relative difference of 0.4% and absolute difference of 0.01 pg, however the haemolymph did not yield results at 3 weeks of storage. Hence, gill tissue can be a preferable tissue for processing as storage till 3 weeks time did not have much affect on the flow cytometry estimation of genome size. In case of pleopod, although the genome size could be estimated till 3 weeks of storage however, the relative difference of 1.11% and absolute difference of 0.03 pg, were higher as compared to gills and haemolymph.

In conclusion, the present study was undertaken to estimate genome size in some of the brackishwater species which will help guiding the research aimed towards generating the sequence data for the whole genome of these species in future. The lesser genome size of *L. calcarifer* (0.44 ± 0.02 pg) obtained in this study when compared to the reported larger genome size of this fish indicated presence of a distinct species in Indian subcontinent. Except in *P. monodon*, significant difference was found in the genome size between the either sex of shrimps with the highest intraspecific relative difference observed in *P. indicus*. The interspecific relative difference was highest between the male shrimps of *P. monodon* and *P. indicus* and between the female shrimps of *P. monodon* and *P. japonicus*. Gills and pleopod tissues of shrimp could be successfully used for genome estimation by flow cytometry till 3 weeks of storage time.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval The fishes used in the experiment during the blood collection, were anesthetized with 2-Phenoxyethanol (Sigma-Aldrich, Co., St. Louis, MO, USA). The blood was collected using 5-ml syringe containing 0.01 M phosphate-buffered saline (PBS) from the caudal vein of the fish. The permission was obtained from the Institute Animal Ethics Committee (IAEC) of ICAR-Central Institute of Brackishwater Aquaculture, Chennai, India to carry out the experiments in fishes. The standard operating procedures in the experiment were followed which were set for the Institutional Animal Ethics Committee by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment Forests and Climate Change, Government of India.

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