



Karyological Analysis of Mud Crab and Flower Crab of Odisha

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Abstract

The present study focuses on the karyological analyses of two commercially important edible crabs namely, *Scylla serrata* and *Portunus pelagicus*. The number and characteristics of chromosomes of these two crabs were studied from spermatogonial metaphase of testes tissues. A total of 56 mitotic metaphases were examined in case of *Scylla serrata* (mud crab). The diploid chromosome number ranged from 99 to 108 with a mode at 106 ($2n=106$), representing 48.2% of the 56 metaphase compliments. A total of 50 mitotic metaphases were studied in *Portunus pelagicus* (flower crab or blue crab). The diploid chromosome number ranged from 92 to 101 with a mode at 98 ($2n=98$), representing 50% of the 50 metaphase compliments. Here sex chromosomes were not detected.

Keywords: Crab, karyology, *Scylla serrata*, *Portunus pelagicus*.

Introduction

Scylla serrata is known as Mud crab, the common edible crab of largest size available in Chilika lagoon. In Odisha this is locally named as REDHA KANKADA. *Portunus pelagicus* is known as flower crab. It represents a valuable component of crustacean fishery. In India, it forms a small scale fishery at different regions along the east and west coasts. In Odisha it is locally named as RANI KANKADA. Karyological studies provides basic information about the number, size and morphology of chromosomes. Since all evolutionary changes have their origin in the chromosomes, any significant alteration in structure, number and behaviour of the chromosomes in the karyotype of a species is also reflected in phenotypic changes which have definite importance in the process of evolution, while morphological features may be influenced by external environmental changes and as such are not so reliable for taxonomic consideration. The crabs are classified under decapod crustaceans, which posses numerous small chromosomes of moreover same shapes which make them difficult to differentiate and count. Great variations in chromosome numbers also have been reported among related species of decapods. For example, two related species of fresh water prawn family Palaemonidae, *Macrobrachium rosenbergii* and *Macrobrachium rude* have 118 and 96 chromosomes respectively while two related species of crabs of Grapsidae family; *Varuna litterata* and *Sesarma tetragonum* have 146 and 94 chromosomes respectively¹.

Cytogenetic study has immense importance in characterising, classifying and drawing evolutionary relationship among the species². In recent years, chromosomal studies in fishes became a priority area of research^{3, 4}. In general, cytogenetic studies of crustaceans are relatively few and very difficult to perform because their chromosome numbers are large⁵⁻¹² and they are of

small dot and rod shaped. Their shapes are very variable such as metacentric, sub metacentric and acrocentric chromosomes¹³⁻¹⁵. Karyological studies were undertaken in an attempt to differentiate between two species of mud crabs namely *Scylla serrata* and *Scylla tranquebarica*¹⁶. The marine shrimp *Penaeus merguiensis* from the Persian Gulf¹⁷ was studied for understanding the geographical differences in chromosome number. By understanding the basic genetic profile of these crabs, genes of commercial importance can be identified and product quality can be improved in terms of consumer preference and human nutrition. The present paper investigates the karyomorphometrical data of these two edible common crabs as karyotype analysis is the most important step towards the gene manipulation and related genetic engineering.

Material and Methods

Five adult males, each of *Scylla serrata* and *Portunus pelagicus* were collected from Satapada, Chilika lagoon ($19^{\circ} 48' N$, $85^{\circ} 49' E$) of Odisha respectively for the present study. After capture, they were transported from the collection sites in live condition in oxygen filled polythene bags and buckets to the laboratory and maintained in aquaria. Testes tissues were dissected out and pretreated with 1% KCl solution and then fixed in freshly prepared Carnoy's 1:3 acetoalcohol fixative. The fixed material was kept in refrigerator for 2 days. Then the tissues were centrifuged for thrice at 3,000rpm for 10 minutes. The fixative was changed for thrice with an interval of 15 minutes. Slides containing chromosome spreads were prepared from the fixed tissues¹⁸. The tissues were stained in 10% Giemsa¹⁹ in Sorenson's phosphate buffer (pH 7) for 25 minutes and rinsed in distilled water. The well spread metaphase plates were observed, analysed and photomicrographed at 100X magnification with an Olympus research microscope coupled with digital camera. In the preparation of karyotypes, the print

of chromosomes of well spread metaphase complements were cut along their boundaries and individual chromosomes were matched in pairs considering their length and morphology. The pairs of chromosomes were arranged in serial order according to their decreasing order of lengths as the chromosomes were gradually seriated and grouped according to chromosome morphology. Then karyotyping was done²⁰ and metrical studies were made according to previous workers.^{21, 22}

Results and Discussion

Out of 56 spermatogonial metaphase plates examined in case of *Scylla serrata*, 27 plates had 106 chromosomes, while 5 had 105, 2 had 104, 4 had 103, 3 had 102, 2 had 101, 3 had 100, 4 had 99, 1 had 107 and rest 5 had 108 chromosomes (table 1 and figure 3(A)). Therefore the diploid chromosome number of this species is $2n=106$ and out of 56 cells the modal diploid cells' percentage was 48.2%. Thus the diploid number of chromosome in this species is $2n = 106$. This is in conformity with the

observations made earlier⁵ and at variance with the report⁷ where there were 94 chromosomes in the diploid count, 12 less than the present number and 102 chromosomes²³. All the chromosomes were acrocentric ones varying from rod to dot shapes (figure 1 A and B). The primary spermatocytic metaphase chromosomes presented a circular arrangement. The chromosomes were highly condensed bodies and were almost round. Out of 55 meiotic spermatocytes metaphase plates examined 21 plates had 53 chromosomes, while 7 had 52, 6 had 51, 3 had 50, 2 had 49, 5 had 48, 4 had 47, 2 had 54, 3 had 55 and rest 2 had 56 chromosomes depicted in table 2 and figure 3(B). As the modal haploid cells' percentage was 38.18% of 55 cells, therefore, haploid chromosome number of this species is $n=53$. The secondary spermatocytes similarly formed smaller circular plates. The chromosomes were round to dot shaped. Thus the chromosome number determined for this species are $2n = 106$ in the diploid and $n = 53$ in the haploid. No chromosomes were observed here as sex chromosomes.

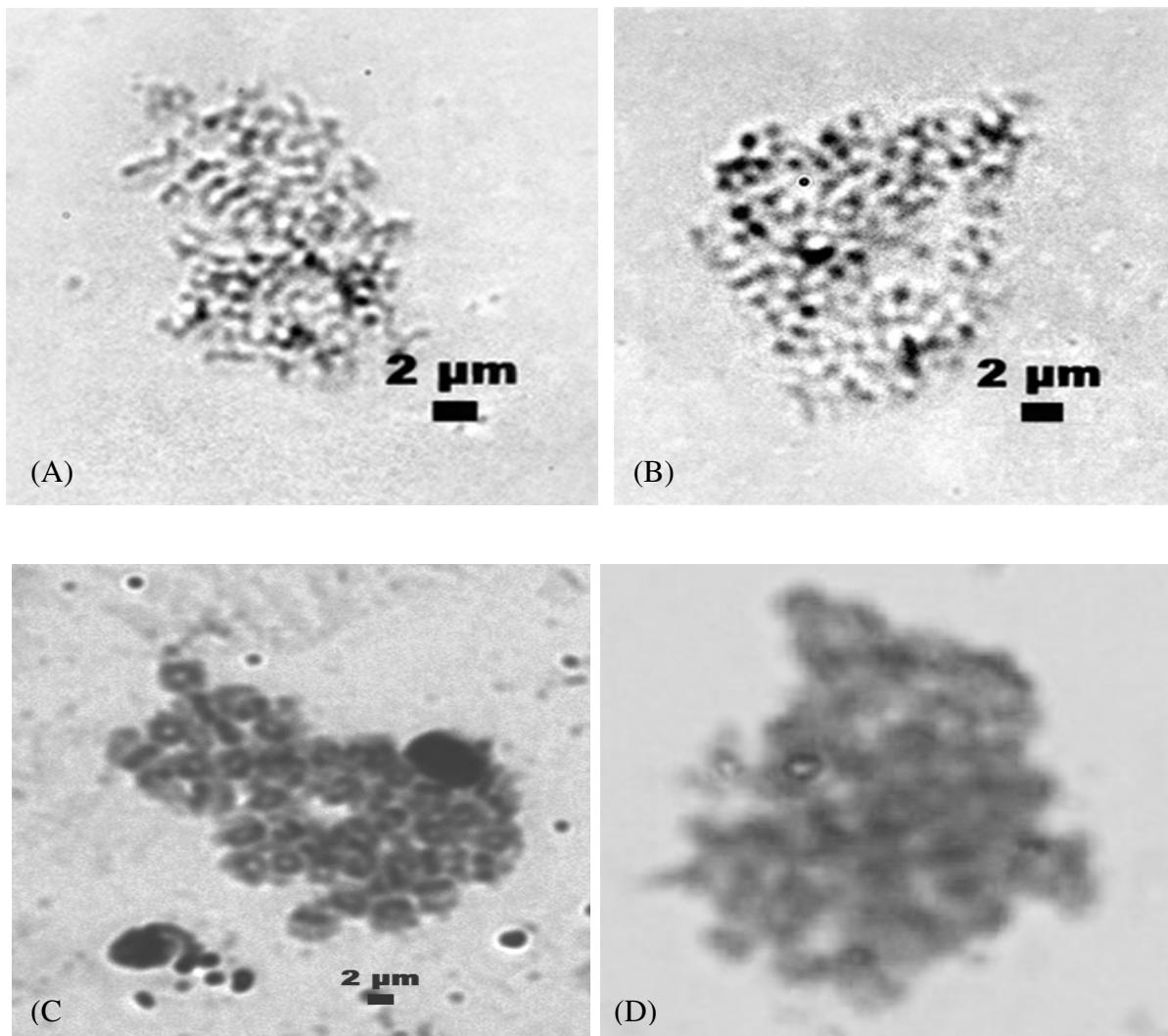


Figure-1
Metaphase complements (A, B) of *S. serrata* and (C, D) of *Portunus pelagicus*.

In *Portunus pelagicus*, out of 50 spermatogonial metaphase compliments examined 25 cells had 98 chromosomes, 3 cells each had 97 and 96, while 2 had 95, 5 had 94, 3 had 93, 4 had 92, 2 had 99, 1 had 100 and rest 2 had 101 chromosomes (table 3 and figure 4(A)). Thus the diploid chromosome number of this species is $2n=98$ as the modal diploid cells' percentage was 50% of 50 cells. The haploid count obtained out of meiotic spermatocyte metaphase compliments revealed 49 chromosomes in 25 cells out of 61 cells studied, while 7 had 48, 6 had 47, 3 had 46, 4 had 45, 6 had 44, 3 had 43, 2 had 50, 3 had 51 and rest 2 had 52 chromosomes. Therefore the haploid chromosome number of this species is $n=49$ as in 61 cells modal haploid cells' percentage was 40.98% (table 4 and figure 4 (B)). The chromosomes were isomorphic-acrocentric in nature and were rods to dot in shape (figure 1C and D). The primary spermatocytic division figures were more numerous. In morphology the metaphase I chromosomes were from round to dots. There was no regular pattern for the arrangement of chromosomes. No chromosome was observed which could be considered as the sex- chromosome in respect to behaviour or morphology. The chromosome sizes varied between 1 to 2 μm in these crabs whereas in amphipod crustaceans it varies 5 to 8 μm ²⁴.

The present comparative karyotypic analysis on *Scylla serrata* and *Portunus pelagicus* report the chromosomes to be almost rod and dot (oval to round) shaped. To establish diploid chromosome number in case of large number of small chromosomes, the numbers observed in maximum metaphasic plates is to be considered¹. Accordingly, the diploid

chromosome number in the mud crab *Scylla serrata* is 106 (figure 2 A and B) while that of flower crab *Portunus pelagicus* is 98 (figure 2 C and D).

The metaphase chromosome counts obtained from testes of *Scylla serrata* and *Portunus pelagicus* have diploid chromosome numbers 106 ($n=53$) and 98 ($n=49$) respectively. The chromosomes of these two species of decapod crustaceans were studied from the male germ cells. Good metaphase plates were obtained from testes. But from gill, hepatopancreas and ovary tissues suitable metaphase plates were not obtained because fat tissue in advanced stages of sexual maturation was hampering the preparation of suitable metaphase chromosomes. The testicular tissues contain both spermatogonias (mitotic cells) and spermatocytes (meiotic cells) for which diploid and haploid chromosome numbers were obtained respectively. Here a very good chromosomal spread obtained by air drying Giemsa technique. The best Giemsa concentration and time for a suitable metaphase plate were 10% and 25 minutes respectively. During dropping of cell suspension the slides also placed on hot plates (43°)²⁵. But in this study chilled slides give better results than hot slides. The use of different concentrations of colchicine reflected no evident difference in the degree of chromosome condensation. However, greater chromosome condensation was obtained due to longer incubation time. This is a study where good metaphasic plates were observed without treatment with chemicals like colchicine. Since the present paper is restricted to chromosome morphology only, therefore, the results of crossing experiments, which do suggest the existence of sex chromosomes in Portunidae family, are not considered here.

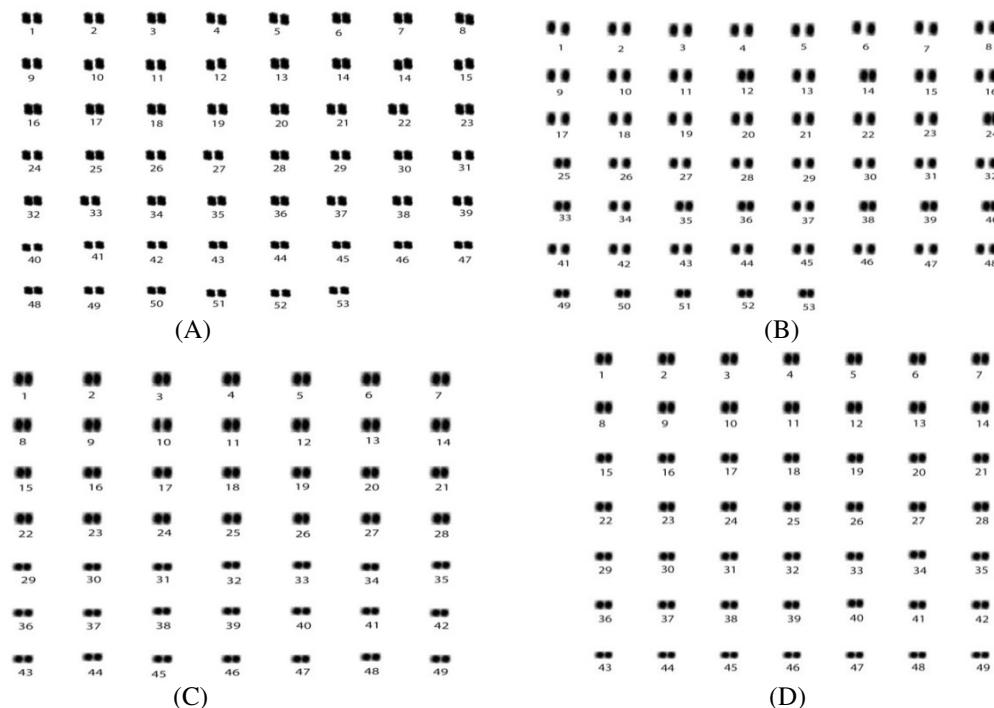


Figure-2
Karyotype (A, B) of *S. serrata* and (C, D) of *P. pelagicus*

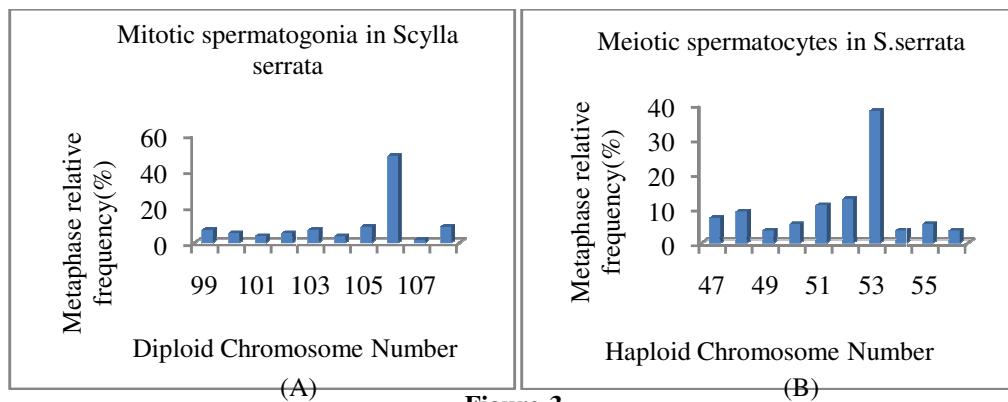


Figure-3
Frequency distribution for diploid and haploid chromosome counts in *S. Serrata*

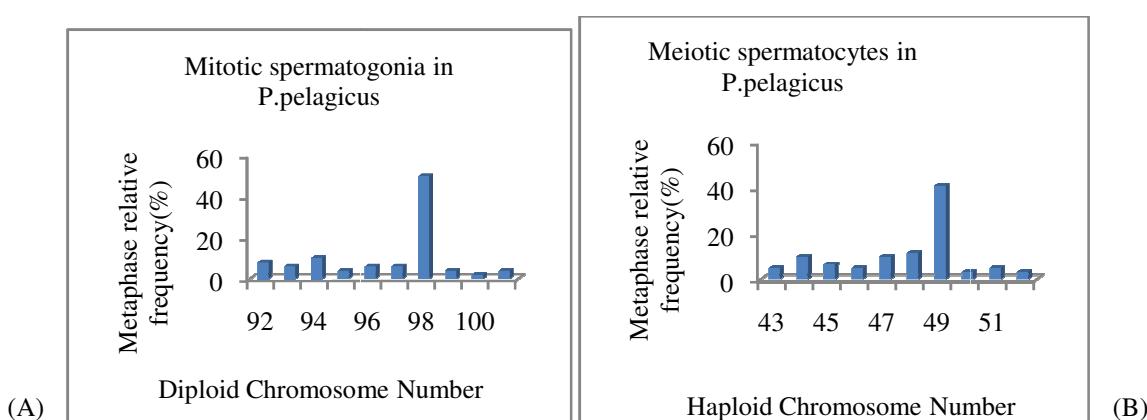


Figure-4
Frequency distribution for diploid and haploid chromosome counts in *P. pelagicus*

Table-1
Diploid chromosome numbers of *Scylla serrata*

No of chromosomes	99	100	101	102	103	104	105	106	107	108	Total
Cells	4	3	2	3	4	2	5	27	1	5	56
Percentage	7.14	5.35	3.57	5.35	7.14	3.57	8.92	48.2	1.8	8.92	100

Table-2
Haploid chromosome numbers of *Scylla serrata*

No of chromosomes	47	48	49	50	51	52	53	54	55	56	Total
Cells	4	5	2	3	6	7	21	2	3	2	55
Percentage	7.27	9.09	3.63	5.45	10.9	12.72	38.18	3.63	5.45	3.63	100

Table-3
Diploid chromosome numbers of *Portunus pelagicus*

No of chromosomes	92	93	94	95	96	97	98	99	100	101	Total
Cells	4	3	5	2	3	3	25	2	1	2	50
Percentage	8	6	10	4	6	6	50	4	2	4	100

Table-4
Haploid chromosome numbers of *Portunus pelagicus*

No of chromosomes	43	44	45	46	47	48	49	50	51	52	Total
Cells	3	6	4	3	6	7	25	2	3	2	61
Percentage	4.91	9.83	6.55	4.91	9.83	11.47	40.98	3.27	4.91	3.27	100

Conclusion

Karyological studies have been undertaken in two species belonging to one family portunidae of decapod crustacean collected from Chilika lagoon. The testes material of the adults exclusively served as the source material for the chromosomal studies. Temporary and permanent squash preparations of the testes were stained respectively with lacto-aceto-orcein and Heidenhain's Iron-haematoxylin and also in air drying Giemsa stain. These three methods were conducted but suitable chromosome plates were obtained in air drying Giemsa technique. Chromosome number, form and size were determined from spermatogonial metaphase counts of these two species. The spermatogonial chromosomes of these species are isomorphic being rod and dot shaped and acrocentric. Minute size and large number of the chromosomes render their counting quite difficult. The diploid chromosome number in *Scylla serrata* and *Portunus pelagicus* was ascertain to be 106 and 98 respectively.

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References

1. Ahmed M.J., Studies on the chromosomes of Decapod Crustaceans of the Chilika Lake, Thesis, Utkal University, Orissa, India (1984)
2. Biswal J.R., Sahoo P.K. and Mohanty P.K., Karyotypic diversity of some freshwater fish species in rural ponds of Orissa, India, *Cytologia*, **75**(3), 237-242 (2010)
3. Barat A., Sahoo P.K. and Ponniah A.G., Karyotype and nucleolar organizer regions (NORs) in a few hill stream fishes. In: Proceedings of The Fifth Indian Fisheries Forum. Ayyappan, S., Jena, J.K. and Mohan, J.M. (Eds.), AFSIB, Mangalore and AoA, Bhubaneswar (2002)
4. Sahoo P.K., Nanda P. and Barat A., Karyotype analysis of *Neolissocheilus hexagonolepis* (McCelland), *Puntius ticto* (Ham.) *P.chola* (Ham.) (Family: Cyprinidae, Pisces), *Cytologia*, **72**, 409-413 (2007)
5. Niiyama H., An unprecedently large number of chromosomes of the crayfish. *Astacus trowbridgii* Stimpson, *Annotationes Zoologicae Japonensis*, **35**(4), 229-233 (1962)
6. Mittal O.P. and Dhall U., Chromosome studies in three species of freshwater decapods (Crustacea). *Cytologia*, **36**, 633- 638 (1971)
7. Vishnoi D.N., Studies on the chromosomes of some Indian crustacean. *Cytologia*, **37**, 43-51 (1972)
8. Nayak B. and Ahmed M.J., Chromosome studies in four species of prawns (Decapoda: Crustacea). *Cytobios*, **60**, 189-196 (1989)
9. Campos-Ramos R., Chromosome studies on the marine shrimps *Panaeus vannamei* and *P. calliforniensis* (Decapoda), *Journal of Crustacean Biology*, **17**, 66-673 (1997)
10. Dumas S. and Campos-Ramos R., Triploidy induction in the pacific white shrimp *Litopenaeus vannamei* (Boone), *Aquaculture Research*, **30**, 621-624 (1999)
11. Zhang X., Fuhua L. and Xiang J., Chromosome behaviour of heat shock induced triploid in *Feneropenaeus chinensis*, *Chinese Journal of Oceanology and Limnology*, **3**, 22-228 (2003)
12. Lee T.H., Naitoh N. and Yamazaki F., Chromosome studies on the mitten crabs *Eriocheir japonica* and *E. Sinensis*, *Fisheries Science*, **70**, 211-214 (2004)
13. Salema H. and Heino T., Chromosome numbers of Fennoscandian glacial relict Crustacea, *Annals of Zoology, Fennici*, **27**, 207-210 (1990)
14. Damrongphol P., Eangchuan N., Ajpru S., Poolsanguan B. and Withyachumarkul B., Karyotype of giant freshwater prawn, *Macrobrachium rosenbergii*, *Journal of The Science Society of Thailand*, **17**, 57-69 (1991)
15. Tan X., Qin J.G., Chen B., Chen L. and Li X., Karyological analyses on red-claw crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae), *Aquaculture*, **234**, 65-76 (2004)
16. Gopikrishna G. and Shashi Shekhar M., Karyological and PCR-RFLP studies of the mud crabs- *Scylla serrata* and *Scylla tranquebarica*, *Journal of The Fisheries Society of Taiwan*, **30**(4), 315-320 (2003)
17. Mansouri S.M., Farahmand H. and Khalilabadi F., Chromosome studies on the marine shrimp *Penaeus (Fenneropenaeus) merguiensis* from the Persian Gulf, *Iranian Journal of Fisheries Sciences*, **10**(4), 753-760 (2011)
18. Kligerman A.D. and Bloom S.E., Distribution of F- bodies, heterochromatin and nucleolar organizers in the genome of the central mud minnow, *Umbra limi*. *Cytogenetics and Cell Genetics*, **18**, 182-196 (1977)
19. Lillie R.D., H.J. Conn's Biological Stains. The Williams and Wilkins Company, USA 9th ed. 606-607 (1977)
20. Novitski E., Human Genetics. Macmillan Publishing Co. Inc., New York, 6-7 (1977)
21. Manna G.K., A study of chromosomes during meiosis in forty - three species of Indian Heteroptera, *Proceedings of Zoological Society of Bengal*, **4**, 1-116 (1951)
22. Harper A.M. and MacDonald M.D., Comparisons of length and area in karyotype analysis of Aphididae, *Canadian Journal of Genetics and Cytology*, **10**, 221- 228 (1968)

- 23.** Mittal O.P. and Pahwa S., Chromosomes of crab, *Scylla serrata*, Forskal (Decapoda), *Chromosomal Information service*, **32**, 14-15 (1982)
- 24.** Natyaganova A.V. and Sitnikova T.Ya., Karyotype of the Baikal amphipod *Polyacanthisca calceolata* Bazikalova, 1937, (Crustacea, Amphipoda), *Chromosome Science*, **15(1+2)**, 43-48 (2012)
- 25.** Röder G., Steinlein C., Schmid M. and Linsenmair K.E., Karyotype and chromosome banding in the Turkish desert woodlouse *Desertellio elongates* (Crustacea, Isopoda, Oniscidea), *Experientia: Interdisciplinary Journal for the Life Sciences*, **49**, 339-341 (1993)