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International Journal of Research in Biomedicine and Biotechnology

Original Article

Cytogenetic analysis of three edible *Cybister* species (Coleoptera: Dytiscidae) from Manipur, India

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Received 24 January 2014; accepted 03 February 2014

Abstract

Cytogenetic analysis of three species of aquatic edible beetles viz., *Cybister sugillatus*, *Cybister tripunctatus* and *Cybister ventralis* were performed using meiotic cells to characterize the diploid chromosome number, sex determining system and chromosome morphology. The spermatogonial metaphase cells revealed same diploid chromosome numbers in both *C. sugillatus* and *C. tripunctatus* with the karyotype formula of 2n=37 (18AA+XO) but differing in chromosome morphology and classification. The Y chromosome element is wanting in case of *C. sugillatus* and *C. tripunctatus.* The sex chromosome in *C. sugillatus* is the smallest chromosome among the all the chromosome whereas it is the largest chromosome in *C. tripuunctatus*. In *C. ventralis*, the sex chromosome system is regulated by XY element with the karyotype formula of 2n=18(8AA+XY). In *C. tripunctatus*, the sex element X, displays a positive heteropycnosis during leptotene and zygotene stages.

Key Words: Diploid chromosome, karyotype, *C. sugillatus*, *C. tripunctatus*, *C. ventralis*

1. Introduction

The members of the family Dytiscidae (Predacious diving beetles) under the order Coleoptera feed vigorously upon almost all invertebrates as well as fish eggs and fry. These beetles generally occupy clean and fresh macrophytic leaves near the bottom along littoral zone. They are active swimmers and swift divers. Adult dytiscids size range from 1.4 to 3.8 mm in length. Although most species are small to medium sized, some adults can attain a length of 37 mm. The hind coxa is very large whereas second and third legs are widely separated. Antennae are very long, thread like with 11 segments. The family Dytiscidae is highly diverse and estimated to include about 4,000 species in over 160 genera. Among the genus, species under the genus *Cybister* are the most well known groups. In India there are about 14 species of *Cybister* (Deepa, 2009).

Manipur is a state laying in the north-east India. In this state, members of the genus *Cybister* are considered as important edible insects because of their high nutritive value. They are regarded as a good source of protein in diet (Shantibala *et al*., 2013). These beetles are catched in group from their habitat like ponds, lakes and small rivers and consumed by roasting. The consumers do not sort out them into specific species. However, it was observed that in a cluster of aquatic beetle catched from water bodies, there are 3 or more species of water beetles. As these species are exhaustively collected from wild for consumption purpose, the species may be extinct in near future. Therefore, for the purpose of proper cataloguing and identification, these species were cytogenetically studied and analyzed. In the meantime, cytogenetic information of these species will be helpful in providing inputs for construction of integrated cytological map with molecular linkage map to reveal the function and structure of the chromosome (Goldsmith *et al.*, 2005). Application of chromosome analysis is expanding because of the advantage of karyotypic analysis as taxonomic characters in both animal and plant (Altunsoy and Kilic, 2010, Arslan *et al*., 2012). Dytiscidae group is not extensively studied cytogenetically except some earlier works (Yadav and Karamjeet, 1980, Voinov, 1903). Cytogenetic reports in *Cybister* from India comprised of the male chromosome complement of 5 species only (Table 1). The present work elucidates the cytogenetic details of three species of *Cybister* species, of which two have been previously reported and one species has been newly described. This study mainly emphasized on the diploid chromosome number, sex determining system and chromosome morphology of the species. These results will allow us to distinguish morphologically similar species in the process of cytogenetic cataloguing.

2. Material and Methods

Insect sampling

Fifteen specimens of each insect species were collected from the ponds as well as from the water submerged paddy

Table 1. Diploid chromosome number of *Cybister* species of India

Species	Chromosome formula	Source
Cybister japonicus	2n=43	Abe *et al*., 2002
Cybister lateromarginalis	2n=22	Serrano & Yadav ,1984
Cybister limbatus	2n=43(21+XO)	Serrano & Yadav ,1984
Cybister sugillatus	2n=43(21+XO)	Yadav *et al*., 1984
Cybister tripunctatus	2n=43(21+XO)	Yadav *et al*., 1984

fields and adjoining areas of Tangkham Lamphel Pat, Khundrakpam, Imphal East district which lies at latitude of 24°50'8.451"N to 24°49'24.695"N and 93°55'38.739"E to 93°55'4.426"E. The beetles were kept alive in aquaria at room temperature for 48 h until they could be processed in the laboratory. They were fed with living aquatic organisms like small fish, snail, tadpole etc.

Cytogenetics

Chromosomes were obtained from testis of adult beetles. Cytological preparations were obtained from the gonads of the adult male individuals. The method of obtaining and preparing the karyotype from beetle tissues was done as described by Shaarawi and Angus (1991) with some slight modification. Hypotonic solution of potassium chloride (0.456 %) and fixative of methanol and glacial acetic acid (3:1) were treated for 30 minutes each. Best results were obtained from 2 day old slides. The slides were stained with 5% Giemsa in phosphate buffer for 10 minutes. The diploid chromosome and characteristic morphology of these species were obtained from 60 chromosome plates from cells exhibiting the complete chromosome number. Images of the selected chromosome plates were taken and karyotypes were prepared by using Leica microsystem Software, CytoVision. Based on the most common classification system used for insect chromosomes, the morphology of chromosomes was determined according to the arms, where the length of the long arm (q) was divided by the length of the short arm (p). Chromosomes were classified as metacentric (m) with arm ratio between 1–1.7, submetacentric (sm) with arm ratio between 1.71-3 and subtelocentric (st) with arm ratio between 3.01-7, whereas chromosomes with a single arm were considered acrocentric (a) with arm ratio above 7. The ideogram was prepared in Harvard Graphics 2.0 software (Esmaeili *et al*., 2010).

3.Results

Metaphase spreads along with some specific chromosomal stages of three species of aquatic edible insects under the genus *Cybister* viz., *C. sugillatus*, *C. tripunctatus* and *C. ventralis* are given in figures 1-3. The meiotic spermatogonial metaphase of *C. sugillatus* gave a diploid chromosome number of 2n = 37(Figure 1). The chromosomal formula of this species is 2n= 37 (18AA+X0). Chromosomes paired according to their length and centromeric positions were arranged into 18 pairs and 1 unpaired sex chromosome. It is characterized in the corresponding idiogram (Figure 4a). The chromosome pairs consist of 10 metacentric, 4 submetacentric and 4 acrocentric chromosomes and a single acrocentric sex chromosome. Centromeric index (CI) and arm ratio (AR) is given in the table 2. The sex determining element in this species is X0 system. The X element is clearly visible in early zygotene stage of the cell (Figure 1a).

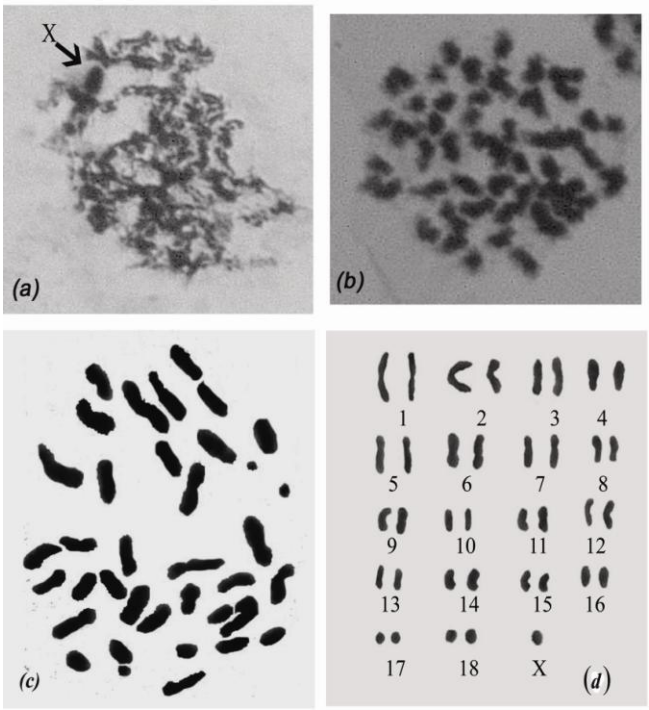

Figure 1: (a) zygotene (b) early metaphase (c) metaphase spread (d) karyotype of *C. sugillatus*

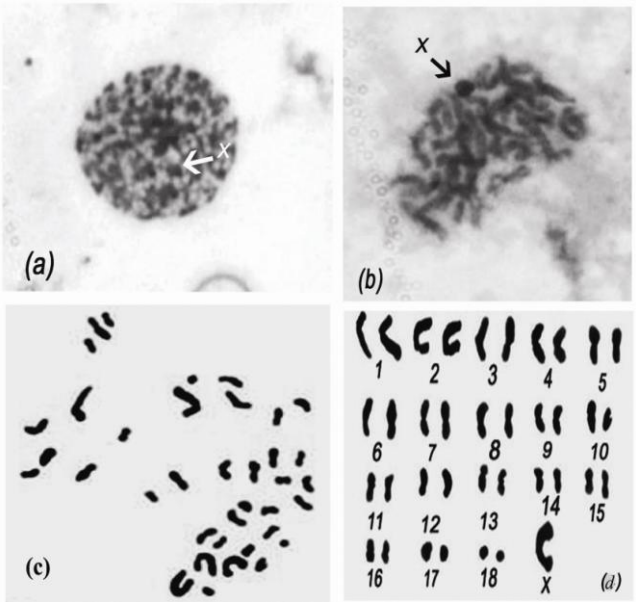

Figure 2: (a) leptotene (b) zygotene (c) metaphase spread(d) karyotype of *C. tripunctatus*

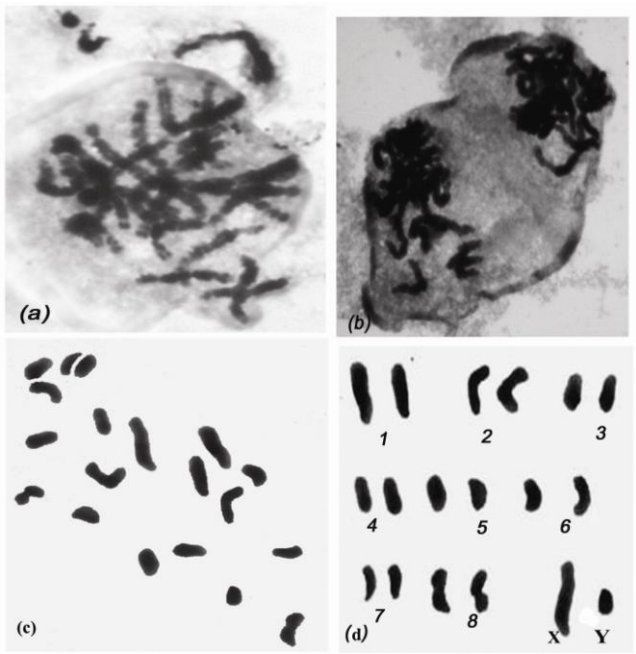

Figure 3: (a) zygotene (b) anaphase (c) metaphase spread (d) karyotype of *C. ventralis*

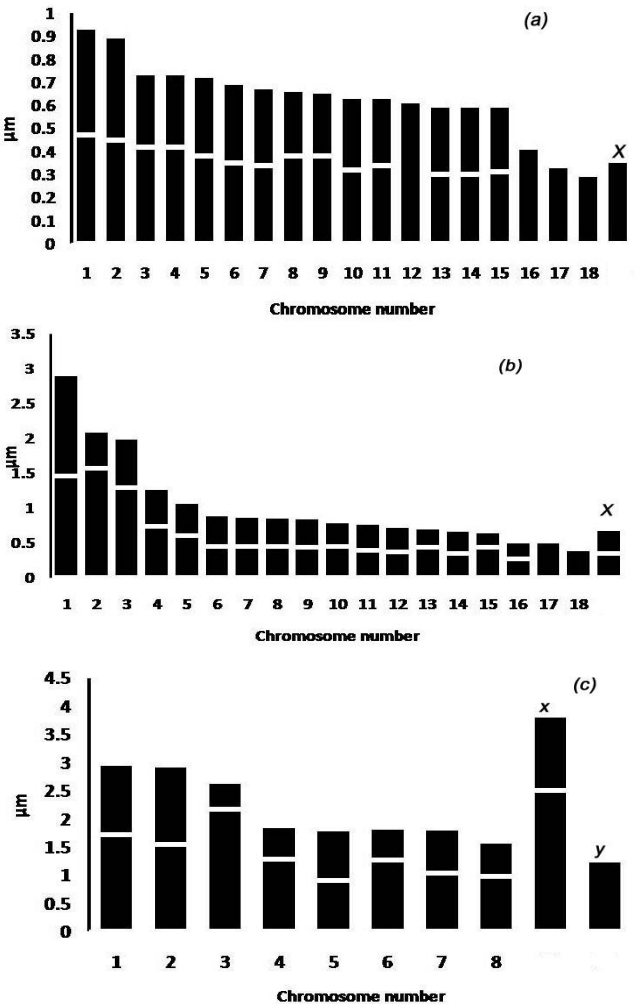

Figure 4. (a) Haploid idiogram of *C. sugillatus* (b) Haploid idiogram of *C. tripunctatus* (c) Haploid idiogram of *C. ventralis*

C. tripunctatus, on the other hand, is one of the most abundant species among the members of the Dytiscidae family recorded during the study. The spermatogonial metaphase revealed 2n= 37 with the chromosome formula 2n =37 (18AA+XO). These chromosomes are differentiated into 14 metacentric, 3 submetacentric, 2 acrocentric autosomes and 1 metacentric X chromosome. Since, idiogram (Figure 4b) showed that majority of the chromosome were metacentric, hence most of the chromosome shows CI of 50% (Table 2). The first 18 bars show the autosome whereas last metacentric X chromosome is the largest chromosome and it is the sex complement. The sex chromosome displays a positive heteropycnosis during leptotene and zygotene stages (Figure 2a & b). The Y chromosome compliment is wanting here.

C. ventralis is by far the largest species among the species of *Cybister*. The karyotype of the spermatogonial metaphase of *Cybister ventralis* gave a diploid chromosome number of 18 pairs with the chromosome formula of 2n=18(8AA+XY) (Figure 2c).Chromosomes were arranged in 9 pairs according to their length and centromere position. It has 8 autosomes and heteromorphic sex element (XY) as shown in the idiogram (Figure 4c). The diploid number includes 5 pairs of metacentric, 1 pair of subtelocentric, 3 pair of submetacentric, and one achrocentric X chromosome and one metacentric Y chromosome.

4. Discussion

The paper emphasizes on the comparative cytogenetic analysis of three species of aquatic edible beetle of the genus *Cybister*. Comparative karyology has some advantage over other methods use in taxonomic studies of insects and other animal. In particular, chromosomal characters are essentially morphological and therefore, they can be analyzed approximately in the same way as other morphological features (Altunsoy and Kilic, 2010). The number of chromosome per cell seems to be a rather conservative characteristic and so may be used as an indicator of the closeness of species interrelationships within families (Moyli and Cech, 2004). In the meantime, within a genus the species tend to have broadly similar karyotypes (Angus and Tatton, 2011). According to the present result, two species viz., *C. tripunctatus* and *C. sugillatus* has same number of diploid chromosome, 2n=37 but markedly differing in karyotypic formula and the sex chromosome. Though the above two species shares the same chromosome number, in order to critically examine the chromosome length and classification (Packiam *et al*., 2013), the morphometric data on chromosome length as well as relative length were calculated. Such report in the chromosome number in these two species was also earlier reported but with different chromosome number of 43 (Yadav *et al*., 1984, Blackmon and Demuth, 2012). The differences in chromosome number obtained from this study might be attributed to the differences in karyotype macrostructure, reflecting a real geographical variation common to widespread species or may be due to degeneration of the autosomal chromosomes in the course of time as the earlier reported work was done years back.

Table 2: Relative length, arm ratio and chromosome type of three aquatic edible beetles.

	C. sugillatus			*C. tripunctatus*			*C. ventralis*		
No.	CI (%)	AR	Type	CI (%)	AR	Type	CI (%)	AR	Type
X	0	1.6±0.62	a	50	1±0.04	m	0	12.73±0.40	a
Y	-	-	-	-	-	-	34	1.93±0.6	sm
1	0	1 ±0.30	a	50	1±0.64	m	49	1.04±0.42	m
2	50	1 ±0.62	m	50	1±0.81	m	39	1.57±0.52	m
3	50	1±0.56	m	50	1±0.45	m	43	1.32±0.09	m
4	0.45	1.22±0.47	m	50	1±0.74	m	32	2.12±0.67	sm
5	50	1±1.29	m	0	4.15±0.67	a	32	2.13±0.32	sm
6	50	1±1.49	m	50	1±0.52	m	47	1.11±0.74	m
7	42	1.4±0.90	m	41	1.44±0.39	m	42	1.41±0.56	m
8	50	1±0.57	m	50	1±0.34	m	20	3.88±0.28	st
9	0	1.4±0.64	a	35	1.8±0.55	sm			
10	33	2±0.47	sm	50	1±0.64	m			
11	31	2±0.51	sm	50	1±0.68	m			
12	45	1.2±0.44	m	45	1.23±0.43	m			
13	0	2±0.98	a	0	5.22±0.57	ac			
14	0	2.2±0.55	a	45	1.24±0.62	m			
15	35	1.83±0.28	sm	44	1.3±0.32	m			
16	35	1.83±0.72	sm	36	1.78± 0.67	sm			
17	50	1±0.85	m		1.00±0.39				
18	50	1±0.51	m		2.91±0.32				

The other species, *C. ventralis* however has a different chromosome number of 2n= 18. It is possible that the reduction in the chromosome number observed in *C. ventralis* might have been due to the involving pericentric inversion followed by fusion between autosomes as in other species of Coleoptera (Bione *et al*., 2005). The chromosomes of the three species have some common character like diminishing size and mediocentric morphology like in *Cicindela* species (Serrano, 1980, Yadav and Karamjeet, 1981 and Yadav *et al*., 1985).

Dytiscidae possess simple sex-chromosome mechanism. In the present study, two species, *C. tripunctatus* and *C. sugillatus* has only X0 chromosome in male individuals like in generalized Dytiscids (Ahmed *et al*., 2000). The present result is in accordance with those of Blackmon and Demuth (2012). The sex chromosome in *C. sugillatus* is the smallest chromosome among the all the chromosome whereas it is the largest chromosome in *C. tripuunctatus*. However the association of XY chromosome system is recorded in the larger *Cybister* species, *C. ventralis*. Such system was also reported earlier in other Dytiscids species like *A. confinis* and *Rantus notatus* (Smith, 1953 and Suorttu, 1971). The heteropycnosis phenomenon of sex chromosome in leptotene and zygotene stages was observed in case of *C. tripunctatus*, such phenomenon was also reported in B-chromosome complement in a locust, *Anacridium aegyptium* (Abdel-Haleem, 2009).

5. Conclusion

Cytogenetic analysis of three species of aquatic edible beetles viz., *Cybister sugillatus*, *Cybister tripunctatus* and *Cybister ventralis* under the family Dytiscidae revealed new karyotype description which is different from the earlier reports. Such cytogenetic analysis of edible insects is very important for proper cataloguing of their chromosome.Further researches are needed on molecular, cytogenetic, anatomical, morphological and biological aspects for better recognition and understanding for the cataloguing of this species so that these bioresources can be used for the better need for human mankind.

Acknowledgements

The authors are thankful to Department of Biotechnology (DBT), New Delhi for providing financial support and working facilities in the Inset Bioresources Division, Institute of Bioresources and Sustainable Development (IBSD), Manipur, India. Further support was also provided to the first author by DBT, through the DBT Research Associateship grant in Biotechnology and Life Sciences program.

References

1. Abdel-Haleem, A.A., Sharaf, H.M., El-Kabbany, A.I.2009. New record of B-chromosome through meiosis in the Egyptian locust *Anacridium aegyptium* (Acrididae) with indication to its origin. Journal of King Saud University (Science) 21:163-166.
2. Abe, A., Obara, Y., Kudoh, K. 2002. Chromosomes of three species of dytiscids (Coleoptera, Dytiscidae). Kontyu 5: 145-154.
3. Ahmed, R.S., Angus, R.B., Aalat, S., Shaarawi, F.2000. Chromosomal analysis of some Egyptian diving beetles (Coleoptera: Dytiscidae). Egyptian Journal of Biology 2:76-84.
4. Altunsoy. F., Kilic, A.,Y.2010. Karyotype characterization of some Tabanidae (Diptera) species. Turkiye Entomoloji Dergisi-Turkish Journal of Entomology 34 (4): 477-494.
5. Angus, R.B., Tatton, A.G.2011. A karyosystematic analysis of some water beetles related to *Deronectes* Sharp (Coleoptera, Dytiscidae). Comparative Cytogenetics 5(3):173–190.

6. Arslan, E., Ertugrul, K., Tugay,O., Dural, H. 2012. Karyological studies of the genus Onobrychis Mill. and the related genera Hedysarum L. and *Sartoria* Boiss & Heldr. (Fabaceae) from Turkey. Caryologia 65(1):11-17.
7. Bione, E., Moura, R.C., Carvalho, R., Souza, M.2005. Karyotype, C- and flourescence banding pattern, Nor location and FISH study of five Scarabaeidae (Coleoptera) species. Genetics and Molecular biology. 28:376-381.
8. Blackmon, H., Demuth, J.P. 2012. Coleoptera Karyotype Database. http://www.uta.edu/karyodb/.
9. Deepa, J.2009. Checklist of aquatic Coleoptera of India, Zoological Survey of India. 16p.
10. Esmaeili, H.R., Zareian, H., Gholamhosseini, A., Ebrahimi, M.2010. Karyotype analysis of the king nase fish, *Chondrostoma regium* (Heckel, 1843) (Actinopterygii: Cyprinidae) from Iran. Turkish Journal Fisheries and Aquatic Science 10: 477–481.
11. Goldsmith, M.R., Shimada,T., Abe, H.2005.The genetics and genomics of the silkworm, *Bombyx mori*. Annual Review of Entomology 50:71-100.
12. Moyli, P, B., Cech, J.J.2004.Fishes. In : An introduction to Ichthyology, 5th ed. Prentice Hall., Englewood cliffs, New Jersey, 726pp.
13. Packiam, S, M., Elumalai, K., Ignacimuthu, S.2013. Karyomorphology, morphometric and ideogram analysis of metaphase chromosome in the oogonial cells of *Spodoptera litura* Fabricius. International Journal of Research in biomedicine and Biotechnology 3(1):1-4.
14. Serrano, J., Yadav, J.S.1984. Chromosome numbers and sex-determining mechanisms in adephagan Coleoptera. Coleopteran Bulletin 38: 335-357.
15. Serrano, J. 1980. *Scarites buparius*, a caraboid beetles with an X_1X_2Y sex-chromosome system. Experientia 36:1042-1043.
16. Shaarawi, F.A., Angus, R.B.1991. A chromosomal investigation of five European species of *Anacaena* Thomson (Coleoptera:Hydrophillidae). Entomologica Scandinavica 21:415-426.
17. Shantibala, T., Lokeshwari, R.K., Gourshyam, Th., Somkuwar, B.G. 2012. Meiman: Database exploring Medicinal and Edible insects of Manipur. Bioinformation 8(10):489-491.
18. Smith, S.G.1953. Chromosome numbers of Coleoptera. Heredity 7:31- 48.
19. Suortti, M.1971. Spermatogenesis in some species of Dytiscidae (Coleoptera). Annales Zoologici Fennici 8: 390-393.
20. Voinov, D.B.1903. La spermatogenese d'ete chez le Cybister roeselli. Archives de Zoologie Experimentale Et Generale 4:173-260.
21. Yadav, J.S., Karamjeet, K.1981.Chromosome studies on three species of Cicindelidae (Adephaga: Coleoptera) from Haryana. Zoologischer Anzeiger Jena 206:121-128.
22. Yadav, J.S., Karamjeet, K.1980. Chromosome number and sex determining mechanism in thirty species of Caraboidea (Adephaga : Coleoptera). Cordulia 6: 20-23.
23. Yadav, J.S., Karamjeet, K., Yadav, A.S.1984. Karyological investigation on seven species of Dytiscidae (Adephaga:Coleoptera) Turkiye Bulteni Kor Dergisi 8:3-16.
24. Yadav, J.S., Kondal, K.,Yadav, A.S.1985. Cytology of *Cicindela (Myriochile) undulata* and *C. (M.) fatidiosa* with a summary of chromosomal data on the Cicindelidae. Cicindela 17:1-11.

Source of support: Nil; Conflict of interest: None declared