



Chromosomal studies on aquatic beetles (Order Coleoptera) from Jammu region

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Abstract: A great number of beetles are adapted to an aquatic existence encompassing a large diversity of habitats and life histories. These belong to families Hydrophilidae and Dytiscidae. During the present investigations, two species of Family Hydrophilidae viz. *Sternolophus rufipes*, *Regimbartia attenuata* and two species of Family Dytiscidae viz. *Dysticus marginalis* (Subfamily Dytiscinae) and *Hydroporus pubescens* (Subfamily Hydroporinae) have been analysed for their chromosome numbers, sex-mechanisms and meiotic behaviour. Aquatic beetles have nutritional value, some economists have investigated the potential for edible insects to provide income and generate jobs for the rural population. Identification is of fundamental importance in diversity studies in a variety of different ways. 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. In spite of many taxonomic studies on aquatic beetles, there has been fewer attempts to explore the karyology of aquatic beetles. Karyological studies provide important information on the genetic structure, life cycles and ecological

characteristics, evolution, taxonomy, and phylogeny of insects. Chromosomal rearrangements can be used for the ecological monitoring of various insect populations and for pest control

Keywords: Hydrophilidae, Dytiscidae, Dabbing, Karyotypes, Bisexual

Introduction:

Only 10% of the 350,000 described species of beetles are aquatic. Aquatic species occur in two major sub-orders, the Adephaga and the Polyphaga. Beetles can be found in nearly any aquatic habitat, but beetles reach their greatest diversity in lentic habitats such as wetlands and pond margins. The reason for their success in aquatic habit is the ability of the adults to enter or leave the water to search for mates or if conditions are not optimal. Aquatic beetles are classified as clingers, climbers, sprawlers, swimmers, divers, and burrowers. Like their habitats, the feeding habits of aquatic beetles are very diverse. Larvae can be herbivores (chewers or piercers), scavengers (gathering collectors), or voracious predators (engulfers or piercers). Many rise to the surface to respire atmospheric oxygen. 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.*, 2012). In general, insects have not made major inroads into salt water, but a considerable number of beetles are able to tolerate such environmental conditions by either physiological tolerance or behavioural adaptation. The Haliplidae (crawling water beetles) are very small beetles living mainly in ponds and the littoral region of lakes. The common species in Europe appear to be related to the trophic characteristics of the lakes that they inhabit, with *Haliphus flavicollus* and *H. fulus* in oligotrophic and *H. heydeni* and *H. ruficollis* in the most eutrophic portion of the lake. The family Dytiscidae is highly diverse and estimated to include about 4,000 species in over 160 genera. They 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. Adults and larvae are aquatic predators.

According to Benazzi (1973), "The karyotype is the fundamental physical basis of evolution, but it is also endowed with evolution of its own, dictated by intrinsic conditions and requirements such as its fundamental mechanism and the balance of gene complex." Understandly, cytogenetics focuses its major attention on the chromosomes. The chromosomes are the nuclear components of special organization, individuality and function which play a vital role in heredity, mutations, variations and evolutionary development of new species. Karyotypes, however, are not stable entities for all time as they undergo several rearrangements which are responsible for bringing out evolutionary changes. Therefore, just as a species has a history, so a

chromosome and a karyotype. White (1963) stated that "evolution is essentially a cytogenetic process". The karyotypic analysis of the species revealed same diploid numbers as reported by earlier workers (Agarwal, 1960a-b; Angus, 1982, 1983, 1986; Manna and Lahiri, 1972, Shaarawi 1989; Yadav and Dange, 1991).

Material and Methods:

The specimens were collected from different areas of Jammu region. The taxonomic position place and period of collection of different Aquatic beetle species worked out during the present study have been listed in the Table-1. Gonads constituted the material for the present investigations. The species have been studied with regard to meiotic chromosomes, karyotypes and sex mechanism. Testicular tissue constituted the material for present study by applying the Air during Geimsa staining technique. The slides were prepared by employing the methods like Squashing, Air- drying, Splashing, Dabbing, Staining, and Scanning of chromosomal slides. Chromosomal slides were stained conventionally with Giemsa stain. The air-dried slides were stained with 2% Giemsa stock solution in phosphate buffer (pH 6.8). After conventional staining, slides were scanned thoroughly under Nikon - Binocular Research Microscope and good metaphase plates were recorded. At least 30-40 well spread Spermatogonial metaphase complements were analysed under 100x oil immersion objective to determine the exact diploid chromosome number (2n) of each species investigated presently. The number was further confirmed from their respective haploid (n) count at meiotic metaphase-I.

Table 1:List of Beetles (Order: Coleoptera; Suborder: Polyphaga) alongwith their Host Plant, Place of Collection and Period of Collection, Chromosomal Formulae and Meioformulae :

Family:Hydrophilidae				Diploid Count and	Meioformulae
Subfamily : Hydrophilinae		Place of Collection	Period of Collection	Chromosomal Formula	
1, <i>Sternolophus rufipes</i> Fabricus	Aquatic Bushes	Rathua Stream, Jammu	June (2014)	2n = 18 7m + 1sm + Xyp	9+Xyp
2. <i>Regimbartia attenuate</i> Fabricus	Aquatic Bushes	Gharana Wetland, Jammu	June (2015)	2n = 18 3m + 5sm + Xyp	9+Xyp
Family: Dytiscidae					
3. Subfamily : Dytiscinae					
<i>Dytiscus marginalis</i>	Aquatic Bushes	Rathua Stream, Jammu	July(2014) (2010)	2n = 36 7m + 9sm + 1st + XY	9+XY
4. Subfamily : Hydroporinae					
<i>Hydroporus pubescens</i>	Aquatic Bushes	Gharana Wetland, Jammu	July(2015) (2010)	2n = 34 8m + 8sm + 1st + XO	9+XO

Results:

The cytological details of the species studied is as follows:

1. *Sternolophus rufipes* Fab.

Classification

Superfamily : Hydrophiloidea
 Family : Hydrophilidae
 Subfamily : Hydrophilinae
 Genus : *Sternolophus*
 Species : *Rufipes*

Karyotypic details

The diploid number of 18 chromosomes was indicated by sufficient number of cells at metaphase-I which possessed 8 autosomal bivalents arranged in the form of rings and rods; the sex bivalent, however, was parachute in configuration. The karyotype consisted of seven pair of metacentric chromosomes (pair no. 1, 2, 3, 5, 6, 7, 8) and one submetacentric pair (pair no. 4) and heteromorphic pair of sex-chromosomes (X and y), whereas X was metacentric, y was submetacentric (Table-2).

The Photokaryotype, (Fig.2) prepared from well spread spermatogonial metaphase plate (Fig.1) showed 18 chromosomes, comprising 8 autosomal pairs (7 metacentric pairs and 1 submetacentric pair) and one sex chromosomal pair (X-metacentric and Y-submetacentric). The fundamental arm number has been calculated to be 36. The diploid chromosomal formula has been worked out to be $7m+1sm+Xyp$.

Histogram (Fig.3) was prepared on the basis of decreasing value of RL% from chromosome pair number 1 to chromosome pair number 8. The sex chromosome pair was placed at the end showing relative length percentage corresponding to its value. Idiogram of chromosomes (Fig.4) represented the

diagrammatic summary of the karyotype to make individual chromosome clearer.

The karyomorphometrical analysis (Table-2) of the spermatogonial metaphase complement revealed the absolute length of autosomes to vary from 6.9 μ m to 3.92 μ m while the 'X' and 'y' measured 5.7 μ m and 3.2 μ m respectively. The first autosomal metacentric pair was largest among the autosomes with an absolute length of 6.9 μ m, TCL % of 13.55% and relative length of 100 %. The last autosomal metacentric pair was smallest among the autosomes, with an absolute length of 3.92 μ m, TCL % of 7.69 % and relative length of 56.81 %. The metacentric X has an absolute length of 5.7 μ m, TCL % of 11.19% and relative length of 82.60 %. The submetacentric y chromosome has an absolute length of 3.2 μ m, TCL % of 6.28 and relative length of 46.37%.

(b) Meiotic details:

- (i) **Metaphase-I:** Nine bivalents (8 autosomal and 1 sex bivalent) were observed, in the form of condensed rods. The arrow indicates the parachute type association of X and y chromosome. (Fig.5&6)
- (ii) **Anaphase –I:** Showed a distribution of chromosomes to their respective poles indicating first meiosis to be reductional for both autosomes and sex chromosome. (Fig. 7)
- (iii) **Metaphase-II (Fig.):** Plate was observed having eight autosomes and X chromosome, the IInd division being equational for both autosomes and sex chromosome. (Fig.8)
- (c) **C- Banding:** In spermatogonial metaphase cells, the centomeric heterochromatin was seen as C-bands in the pericentromeric region. (Fig.9 and 10).

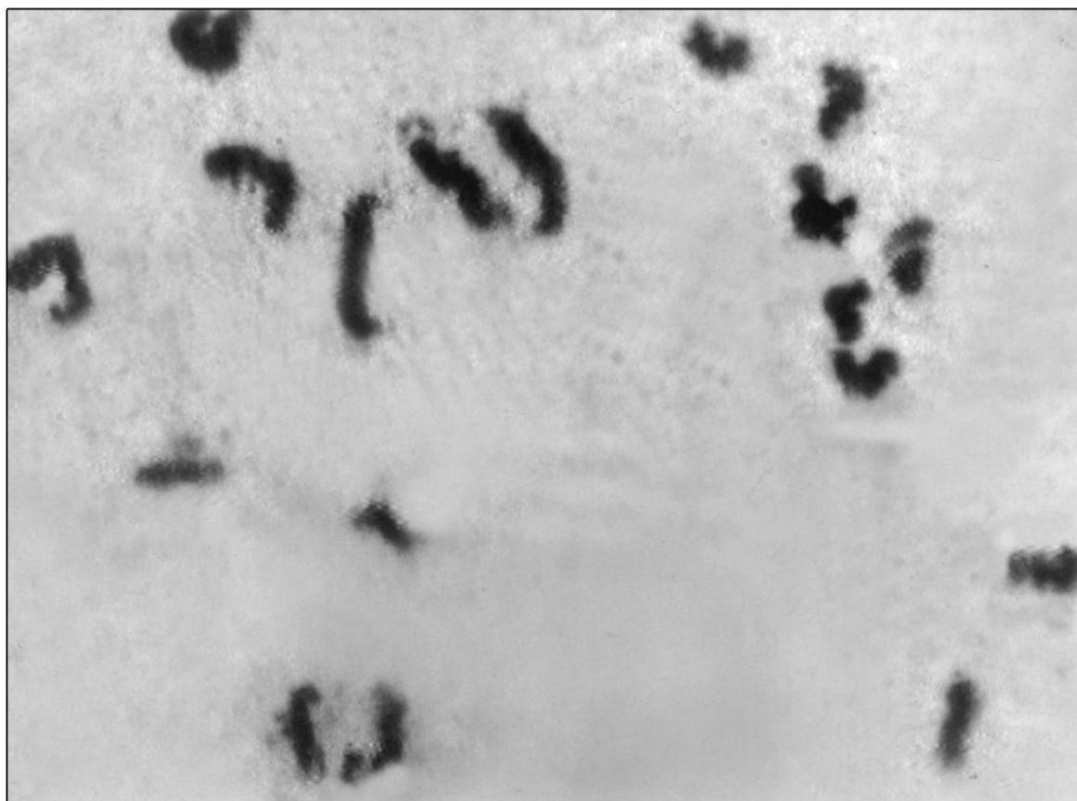


Fig. 1 Spermatogonial metaphase complement of male *Sternolophus rufipes* Fabricus, (2n=18).

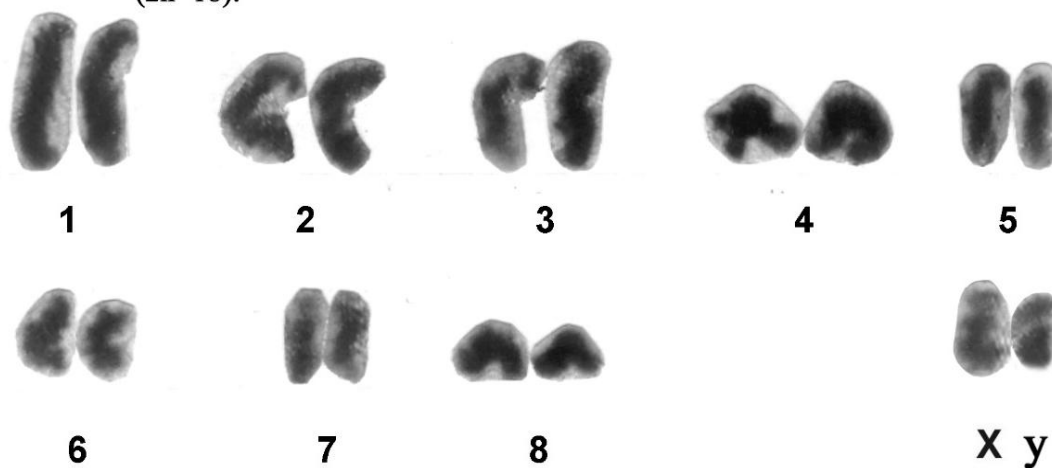


Fig. 2 Photokaryotype of the spermatogonial metaphase complement (7m+1sm+Xyp).

Table 2: Karyomorphometric analysis of Spermatogonial metaphase complement of Male *Sternolophus rufipes*.

Chr. Pair No.	Mean Length of the Short arm (p) in μm	Mean Length of the Long arm (q) in μm	Absolute Length (p + q) in μm	Relative Length (%)	Arm Ratio (AR)	Centromeric Index (CI)	TCL (%)	Nomenclature
1.	3.0	3.9	6.9	100	1.30	43.47	13.55	Metacentric
2.	2.9	3.2	6.1	88.40	1.10	47.54	11.97	Metacentric
3.	2.3	3.5	5.8	84.05	1.52	47.54	11.97	Metacentric
4.	1.9	3.5	5.4	78.26	1.84	35.18	10.60	Submetacentric
5.	2.2	3.0	5.2	75.36	1.36	42.30	10.21	Metacentric
6.	1.9	2.8	4.7	68.11	1.46	40.42	9.23	Metacentric
7.	1.6	2.4	4.0	57.97	1.50	40.00	7.85	Metacentric
8.	1.72	2.2	3.92	56.81	1.27	43.87	7.69	Metacentric
X	2.7	3.0	5.7	82.60	1.11	47.36	11.19	Metacentric
y	0.9	2.3	3.2	46.37	2.5	28.12	6.28	Submetacentric

Total mean haploid length
 Nombre Fondamentale
 Length of X chromosome
 = 50.92 μm
 = 36
 = 5.7 μm

Total mean diploid length
 Sex mechanism
 Length of y chromosome
 = 101.8 μm
 = Xyp(σ^7)
 = 3.2 μm

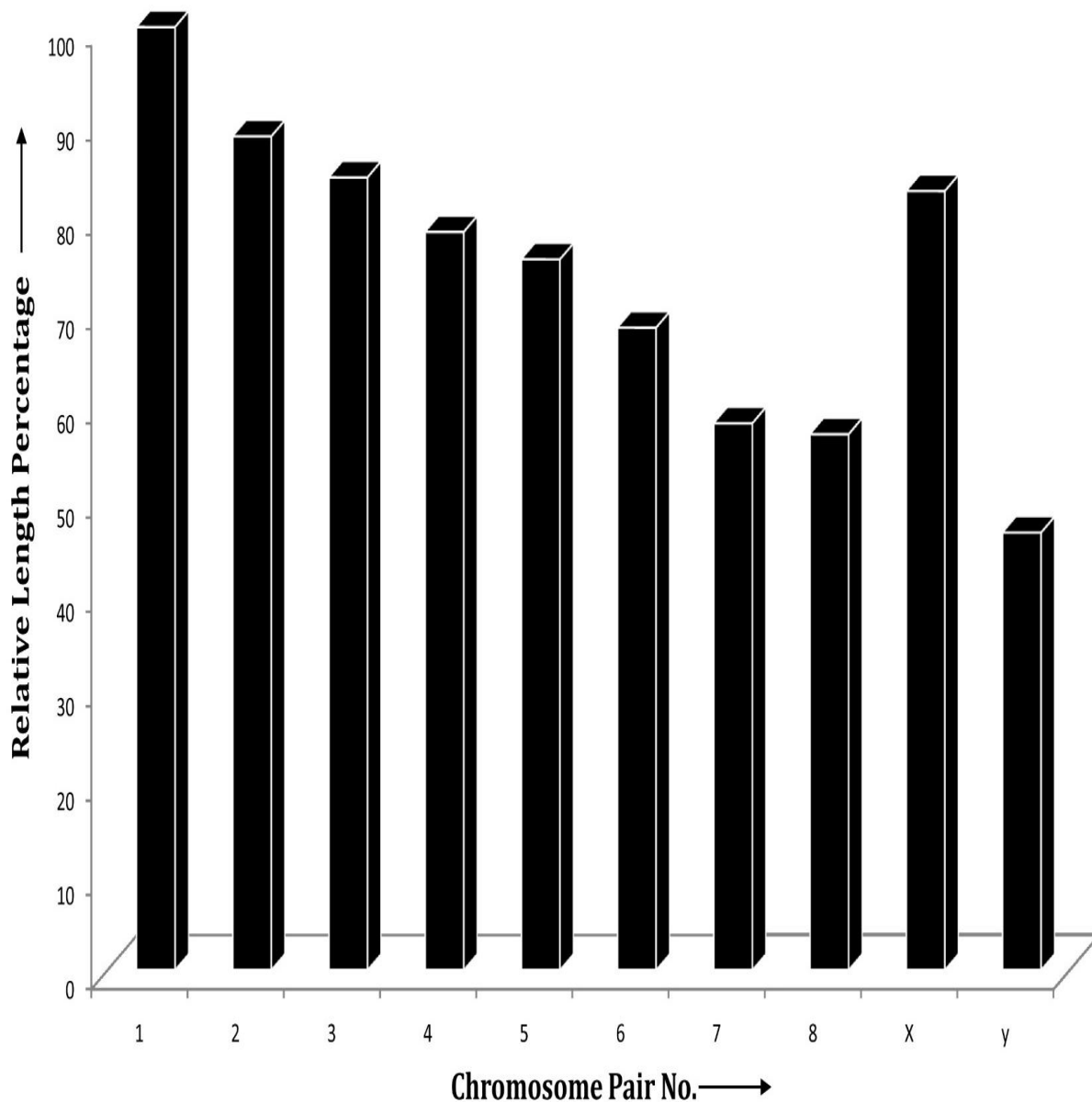


Fig. 3 Histogram of male *Sternolophus rufipes* Fabricus.

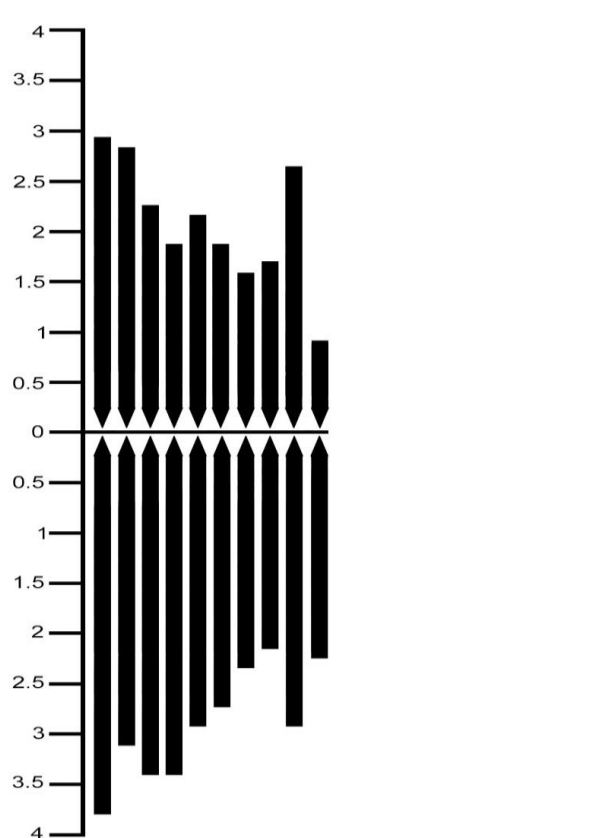


Fig. 4 · An idiogram of male *Sternolophus rufipes* Fabricius.

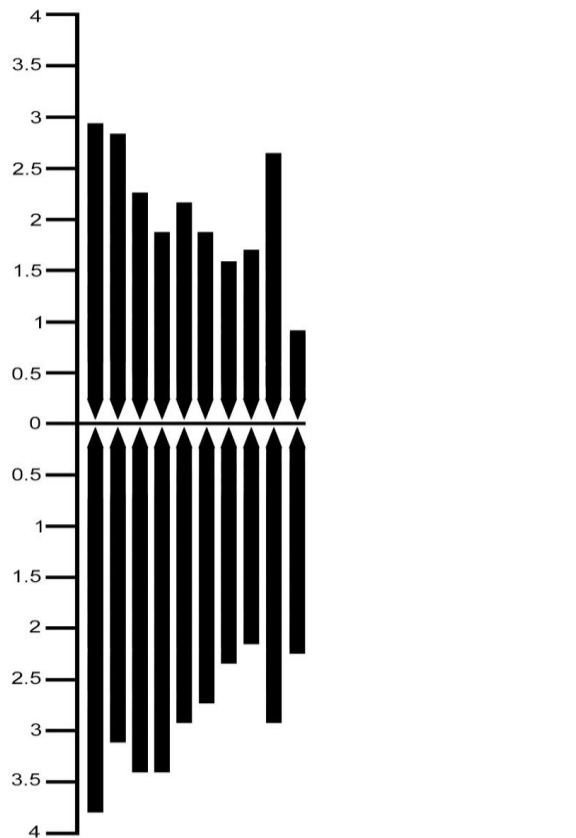


Fig. 4 · An idiogram of male *Sternolophus rufipes* Fabricius.

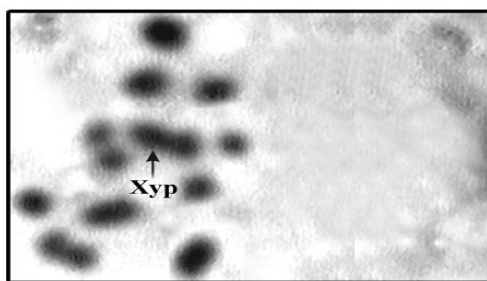


Fig. 5 Metaphase - I

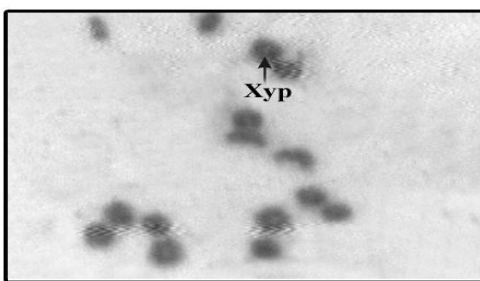


Fig. 6 Metaphase - I

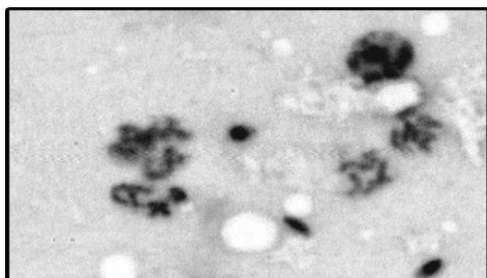


Fig. 7 Anaphase - I

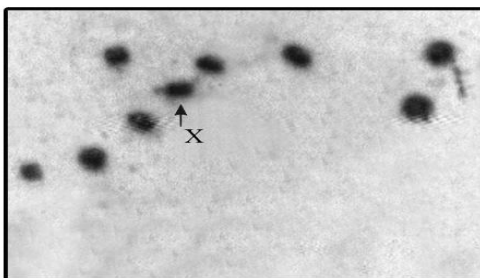


Fig. 8 Metaphase - II

2. *Regimbartia attenuata* Fabr.

Classification

Superfamily	: Hydrophiloidea
Family	: Hydrophilidae
Subfamily	: Berosinae
Genus	: <i>Regimbartia</i>
Species	: <i>Attenuate</i>

(a) Karyotypic details:-

The photokaryotype, (Fig.10) prepared from well spread spermatogonial metaphase plate (Fig.9)(i) showed 18 chromosomes, comprising 8 autosomal pairs (3 metacentric and 5 submetacentric) and one sex chromosomal pair (X-submetacentric and y-subtelocentric). The fundamental arm number has been calculated to be 36. The diploid chromosomal(ii) formula has been worked out to be $3m+5sm+Xyp$.

Histogram (Fig.11) was prepared on the basis of decreasing value of RL% from chromosome pair number 1 to chromosome pair number 8. The sex chromosome pair was placed at the end showing(iii) relative length percentage corresponding to its value. Idiogram of chromosome (Fig.12) represented the diagrammatic summary of the Karyotype to make individual chromosome clearer.

The karyomorphometrical analysis (Table-3) of the spermatogonial metaphase complement revealed the absolute length of autosomes to vary from 5.5µm to 3.45 µm, while the X and Y measured

4.6 µm and 2.6µm respectively. The first autosomal metacentric pair was largest among the autosomes with an absolute length of 5.5µ m, TCL % of 12.07 % and relative length of 100%. The last autosomal submetacentric pair was smallest among the autosomes, with an absolute length of 3.45µ m, TCL % of 7.57 % and relative length of 62.72%. The submetacentric X chromosomes has an absolute length of 4.6 µm, TCL% of 10.10% and relative length percentage of 83.63. The subtelocentric y-chromosome has an absolute length of 2.6 µm, TCL% of 5.79 and relative length percentage of 48.

(b) Meiotic details:

Metaphase-I: Nine bivalents were observed which included 8 autosomal, dumb-bell shaped bivalents and Xyp sex bivalent. The arrow indicates the parachute type association of X and Y chromosomes. (Fig. 13)

Anaphase-I : Showed a distribution of 8 autosomes and the, S, to one pole and 8 autosomes and the y, to the other pole, indicating 1st meiosis to be reductional for both autosomes and the sex chromosomes. (Fig. 14)

Metaphase-I : Two kinds of metaphases-II cells were encountered, one with 8 autosomes and a larger X-chromosomes and the other with 8 autosomes and a small y-chromosome. (Fig. 15&16)

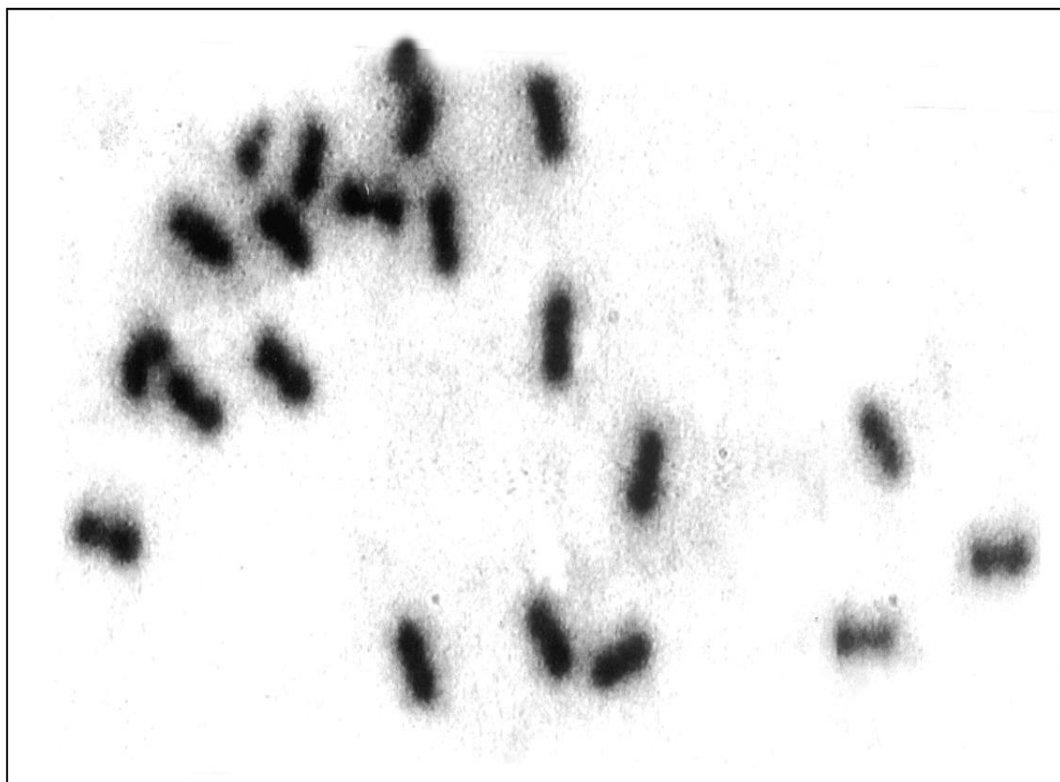


Fig. 9 | Spermatogonial metaphase complement of male *Regimbartia attenuata* Fabricus, ($2n=18$).

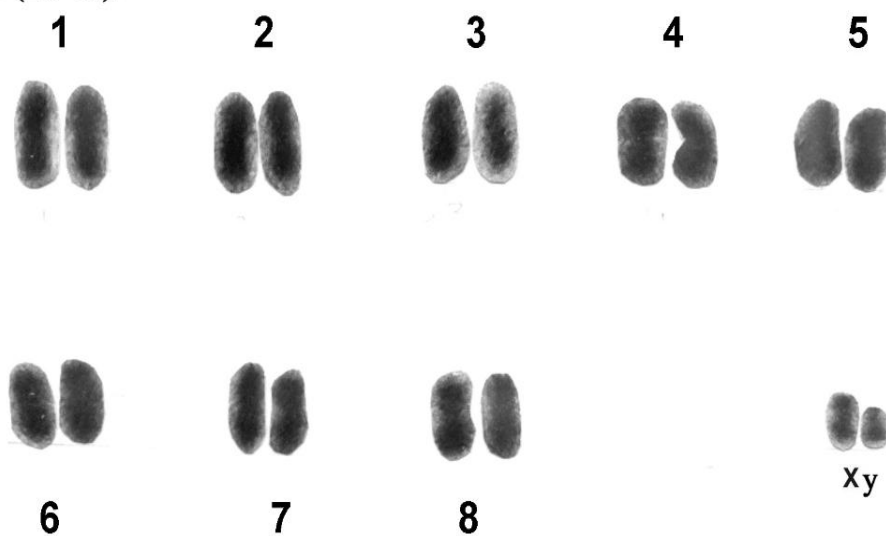


Fig. 10 Photokaryotype of the spermatogonial metaphase complement ($3m+5m+Xyp$).

Table 3: Karyomorphometric analysis of Spermatogonial metaphase complement of Male *Regimbartia attenuata*.

Chr. Pair No.	Mean Length of the Short arm (p) in μm	Mean Length of the Long arm (q) in μm	Absolute Length (p + q) in μm	Relative Length (%)	Arm Ratio (AR)	Centromeric Index (CI)	TCL (%)	Nomenclature
1.	2	3.5	5.5	100	1.75	36.36	12.07	Submetacentric
2.	2.5	2.9	5.4	98.18	1.16	46.29	11.86	Metacentric
3.	2.1	3.1	5.2	94.54	1.29	38.18	11.42	Metacentric
4.	1.87	3.2	5.07	92.18	1.71	26.88	11.13	Submetacentric
5.	1.9	2.8	4.7	85.45	1.47	40.42	10.32	Metacentric
6.	1.67	2.9	4.57	83.09	1.73	36.54	10.03	Submetacentric
7.	1.6	2.8	4.4	80.0	1.75	36.36	9.66	Submetacentric
8.	1.2	2.25	3.45	62.72	1.870	34.78	7.57	Submetacentric
X	1.6	3.0	4.6	83.63	1.87	34.78	10.10	Submetacentric
y	0.4	2.24	2.64	48.0	5.6	15.15	5.79	Subtelocentric

Total mean haploid length = $45.53\mu\text{m}$
 Nombre = 36
 Fondamentale
 Length of X = $4.6\mu\text{m}$
 chromosome

Total mean diploid length = $91.06\mu\text{m}$
 Sex mechanism = Xyp(σ)
 Length of y = $2.64\mu\text{m}$
 chromosome

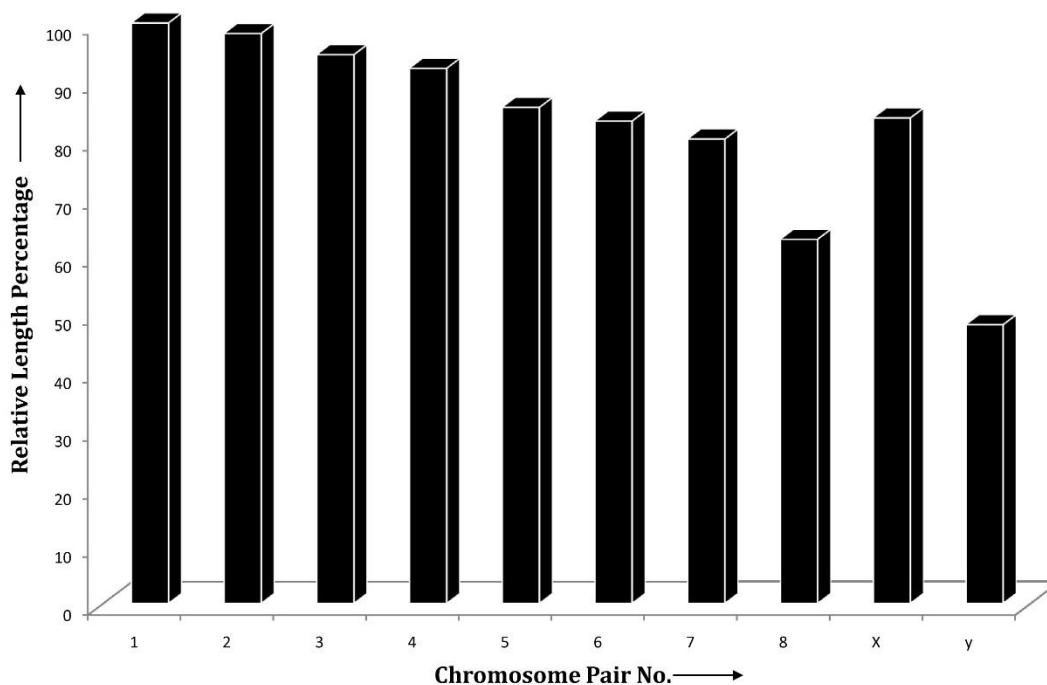


Fig. 11 Histogram of male *Regimbartia attenuate* Fabricus.

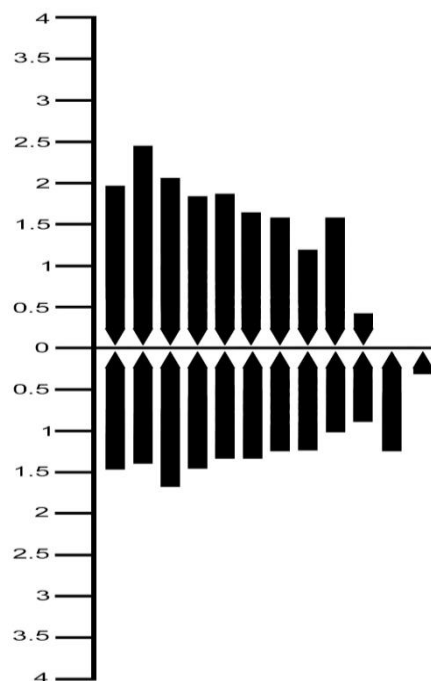


Fig. 12 An Idiogram of male *Regimbartia attenuate* Fabricus.



Fig. 13 Metaphase - I

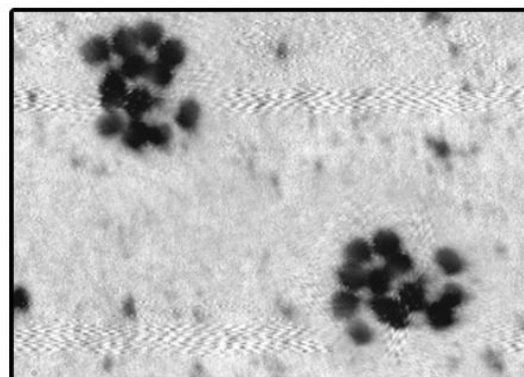


Fig. 14 Anaphase - I

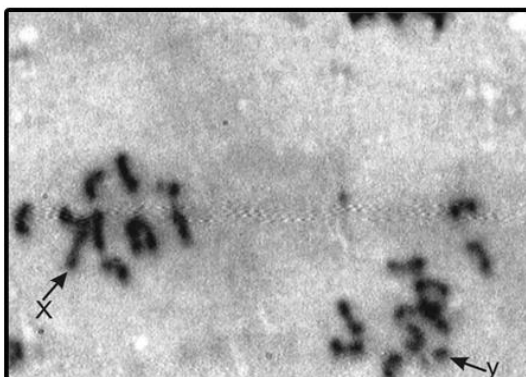


Fig. 15 Metaphase - II



Fig. 16 Metaphase - II

3. *Dytiscus marginalis*

Classification

Superfamily :
 Family : Dytiscidae
 Subfamily : Dytiscinae
 Genus : *Dytiscus*
 Species : *Marginalis*

(a) Karyotypic details:

The photokaryotype (Fig.18) prepared from well spread spermatogonial metaphase plate (Fig.17) showed 38 chromosomes, comprising 18 autosomal pairs (6 metacentric pairs, 10 submetacentric pairs and 2 subtelocentric pairs) and one sex chromosomal pair (X-submetacentric and Y-subtelocentric). The fundamental arm number calculated to be 80. The

diploid chromosomal formula has been worked out to be $6m+10sm+2st+XY$.

Histogram (Fig.19) was prepared on the basis of decreasing value of RL% from chromosome pair number 1 to chromosome pair number 18. The sex chromosome pair was placed at the end showing relative length percentage corresponding to its value. Idiogram of chromosomes (Fig.20) represented the diagrammatic summary of the karyotype to make individual chromosomes clearer.

The karyomorphometrical analysis (Table-4) of the spermatogonial metaphase complement revealed the absolute length of autosomes to vary from $5.13\mu m$ to $1.71\mu m$, while the X and Y measured $5.38\mu m$ and $4.79\mu m$ respectively. The first autosomal submetacentric pair was largest among the autosomes, with an absolute length of $5.13\mu m$, TCL% of 7.55 and relative length percentage of

95.35. The last autosomal Subtelocentric pair was smallest among the autosomes with an absolute length of 1.71, TCL% of 2.52% and relative length percentage of 31.78. The submetacentric X was the largest in the complement, with an absolute length of 5.38 μ m, TCL% of 7.92 and relative length percentage of 100. The Subtelocentric Y-chromosome has an absolute length of 4.79 μ m, TCL of 7.05 and relative length percentage of 89.03.

(b) Meiotic details:

Metaphase-I: Nineteen bivalents (18 autosomal and 1 sex bivalent) were observed in the form of condensed rods. The arrow indicates the parachute type association of 'X' and 'Y' chromosomes. (Fig.21 and 22)

Anaphase-1: A cell at Anaphase-1 was observed having 19 chromosomes at one pole and 19 chromosomes to the other pole indicating first meiosis to be reductional for both autosomes and sex chromosomes. (Fig.23)

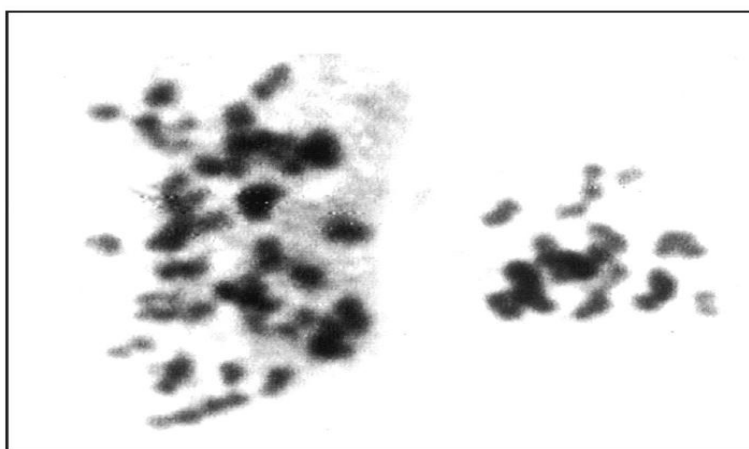


Fig. 17 Spermatogonial metaphase complement of male *Dytiscus marginalis*, ($2n=36+XY$)

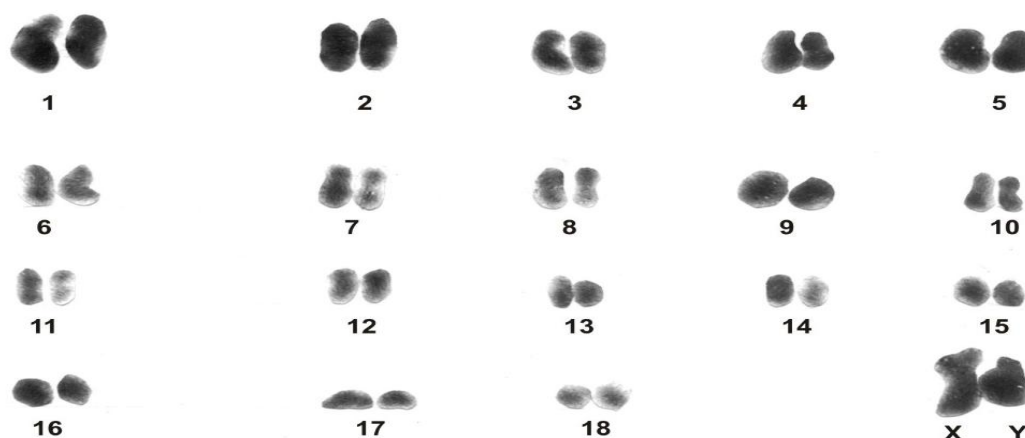


Fig. 18 Photokaryotype of the spermatogonial metaphase complement ($7m+9sm+1st+XY$).

Table 4: Karyomorphometric analysis of Spermatogonial metaphase complement of Male *Dytiscus marginalis*.

Chr. Pair No.	Mean Length of the Short arm (p) in μm	Mean Length of the Long arm (q) in μm	Absolute Length (p + q) in μm	Relative Length (%)	Arm Ratio (AR)	Centromeric Index (CI)	TCL (%)	Nomenclature
1.	1.88	3.25	5.13	95.35	1.73	38.59	7.55	Submetacentric
2.	1.25	2.90	4.15	77.14	2.32	30.12	6.17	Submetacentric
3.	1.48	2.63	4.11	76.39	1.77	36.00	6.05	Submetacentric
4.	1.12	2.46	3.58	66.54	2.19	31.28	5.27	Submetacentric
5.	1.32	2.13	3.45	64.13	1.62	38.26	5.08	Metacentric
6.	1.42	2.02	3.44	63.94	1.42	41.28	5.06	Metacentric
7.	1.34	2.10	3.44	63.94	1.57	38.95	5.06	Metacentric
8.	1.23	2.20	3.43	63.75	1.78	35.86	5.05	Submetacentric
9.	1.47	1.90	3.37	62.64	1.29	43.62	4.96	Metacentric
10.	0.89	2.33	3.22	59.85	2.62	27.64	4.74	Submetacentric
11.	1.25	1.84	3.09	57.44	1.47	40.45	4.55	Metacentric
12.	1.12	1.90	3.02	56.13	1.69	37.08	4.45	Metacentric
13.	0.92	1.89	2.81	52.23	2.05	32.74	4.14	Submetacentric
14.	0.84	1.97	2.81	52.23	2.26	29.89	4.14	Submetacentric
15.	0.82	1.85	2.67	49.63	2.26	30.71	3.93	Submetacentric
16.	0.82	1.46	2.28	42.38	1.78	35.96	3.35	Submetacentric
17.	0.41	1.62	2.03	37.73	3.95	20.19	2.99	Subtelocentric
18.	0.39	1.32	1.71	31.78	3.38	22.81	2.52	Subtelocentric
X	1.45	3.93	5.38	100	2.71	26.95	7.92	Submetacentric
y	0.89	3.90	4.79	89.03	4.38	18.58	7.05	Subtelocentric

Total mean haploid length
 Nombre Fondamentale
 Length of X chromosome

= 67.91 μm
 = 80
 = 5.38 μm

Total mean diploid length
 Sex mechanism
 Length of y chromosome

= 135.82 μm
 = Xyp(σ)
 = 4.79 μm

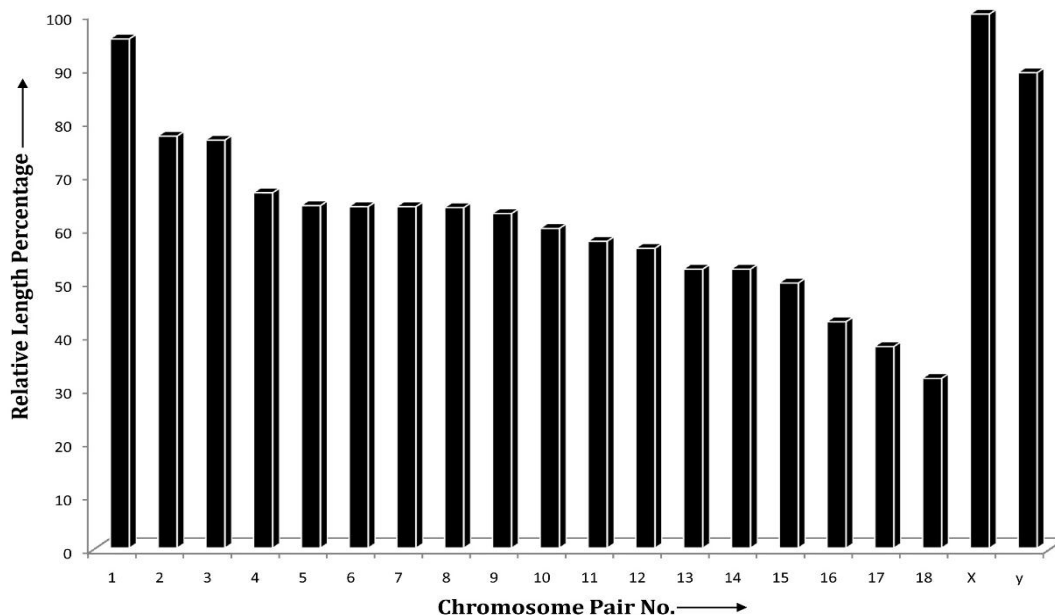


Fig. 19 Histogram of male *Dytiscus marginalis*.

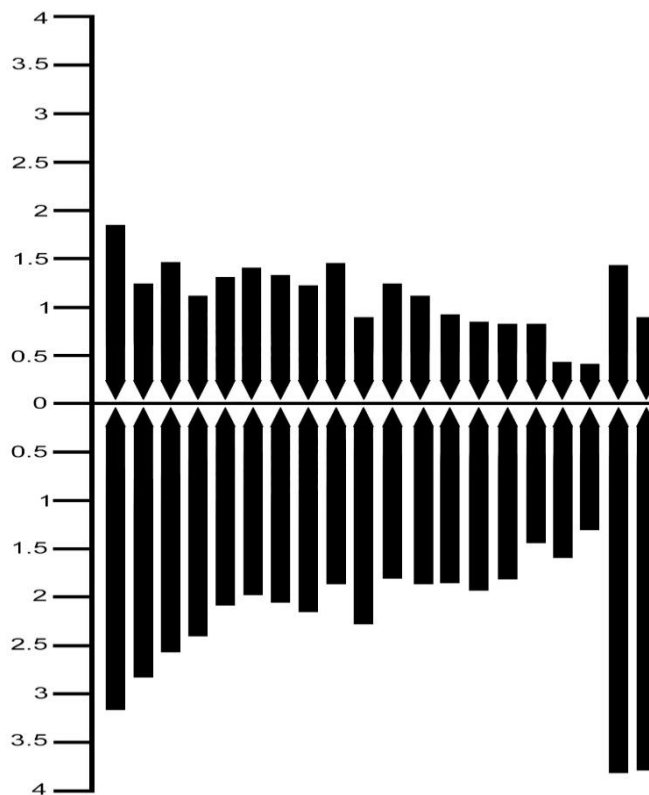


Fig. 20 An Idiogram of male *Dytiscus marginalis*.

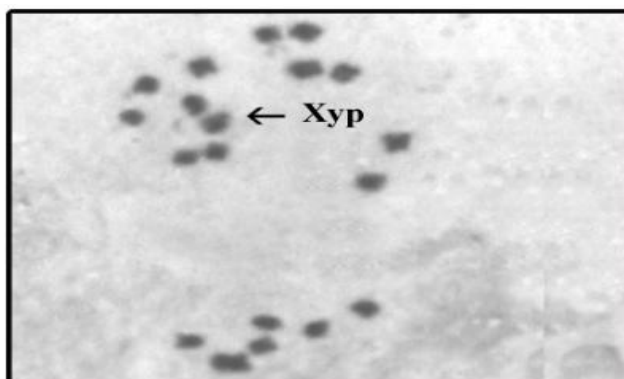


Fig. 21 Metphase - I

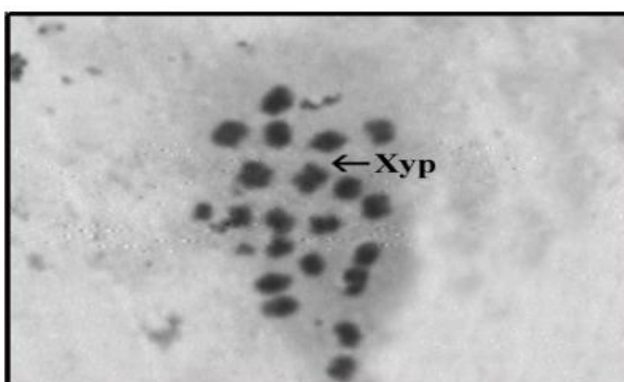


Fig. 22 Metphase - I

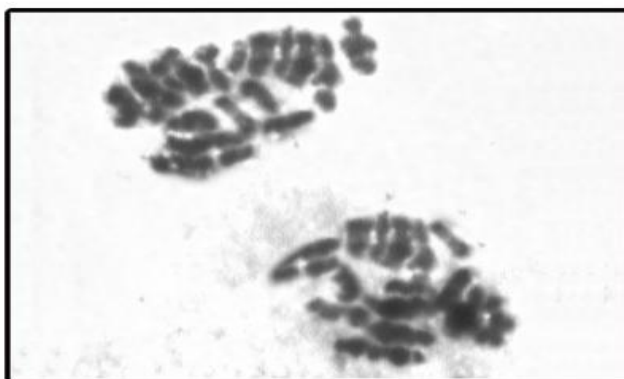


Fig. 23 Anaphase - I

4. *Hydroporus pubescens*

Taxonomic Status

Superfamily : Dytiscoidea
 Family : Dytiscidae
 Subfamily : Hydroporinae
 Genus : *Hydroporus*
 Species : *Pubescens*

(a) Karyotypic details:

The photokaryotype (Fig.25) prepared from well spread spermatogonial metaphase plate (Fig.24) showed 35 chromosomes, comprising 17 autosomal pairs (8 metacentric pairs, 8 submetacentric pairs and 1 subtelocentric), and one sex chromosome (X- the largest metacentric chromosome). The fundamental arm number calculated to be 72. The diploid chromosomal formula has been worked out to be $8m+8sm+1st+XO$.

Histogram (Fig.26) was prepared on the basis of decreasing value of RL% from chromosome pair number 1 to chromosome pair number 17. The sex chromosome pair was placed at the end showing relative length percentage corresponding to its value. Idiogram of chromosomes (Fig.27) represented the diagrammatic summary of the karyotype to make individual chromosomes clearer.

The karyomorphometrical analysis (Table-5) of the spermatogonial metaphase complement revealed the absolute length of autosomes to vary

from $4.00\mu\text{m}$ to $1.44\mu\text{m}$, while the X measured $4.07\mu\text{m}$. The first autosomal metacentric pair was largest among the autosomes, with an absolute length of $4.00\mu\text{m}$, TCL% of 9.12 and relative length of 98.28%. The last autosomal Subtelocentric pair was smallest among the autosomes, with an absolute length of $1.44\mu\text{m}$, TCL% of 3.28 and relative length of 35.38%. The metacentric X was the largest in the complement with an absolute length of $4.07\mu\text{m}$, TCL% of 9.27 and relative length of 100%.

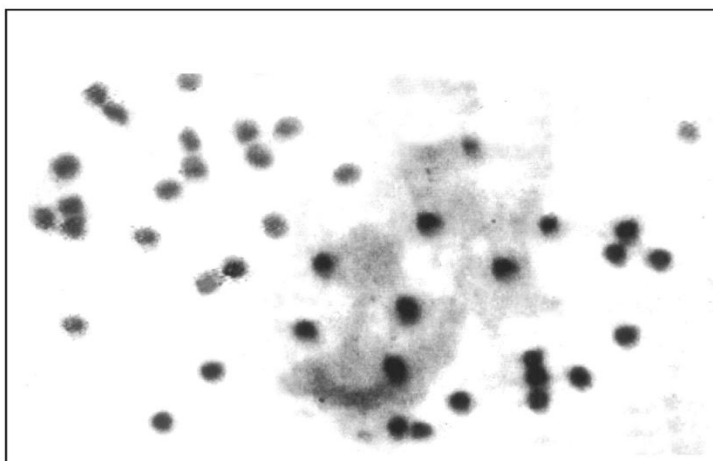


Fig. 24 Spermatogonial metaphase complement of male *Hydroporus pubescens*, ($2n=34+X0$).

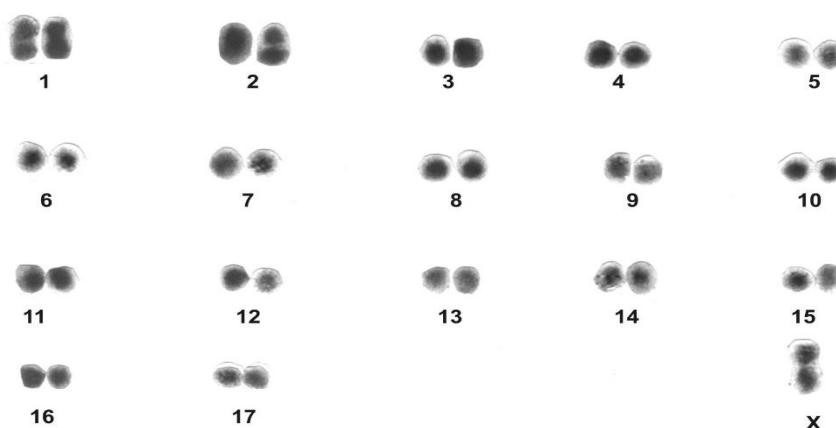


Fig. 25 Photokaryotype of the spermatogonial metaphase complement ($8m+8sm+1st+X0$).

Table 5: Karyomorphometric analysis of Spermatogonial metaphase complement of Male *Hydroporus pubescens*.

Chromosome Pair No.	Mean Length of the Short arm (p) in μm	Mean Length of the Long arm (q) in μm	Absolute Length (p + q) in μm	Relative Length (%)	Arm Ratio (AR)	Centromeric Index (CI)	TCL (%)	Nomenclature
1.	1.83	2.17	4.00	98.28	1.18	45.75	9.12	Metacentric
2.	1.52	1.89	3.41	83.78	1.24	44.57	7.77	Metacentric
3.	0.73	1.82	2.55	62.65	2.49	28.62	5.81	Submetacentric
4.	1.09	1.42	2.51	61.67	1.31	43.43	5.72	Metacentric
5.	1.08	1.43	2.51	61.67	1.33	43.03	5.72	Metacentric
6.	0.82	1.67	2.49	61.18	2.04	32.93	5.67	Submetacentric
7.	0.89	1.57	2.46	60.44	1.76	36.18	5.61	Submetacentric
8.	0.62	1.79	2.40	58.97	2.88	25.83	5.47	Submetacentric
9.	1.02	1.29	2.31	56.76	1.26	44.15	5.26	Metacentric
10.	0.74	1.48	2.22	54.55	2.00	33.33	5.06	Submetacentric
11.	0.76	1.42	2.18	53.56	1.87	34.86	4.96	Submetacentric
12.	0.99	1.19	2.18	53.56	1.21	45.42	4.96	Metacentric
13.	0.83	1.18	2.01	49.38	1.42	41.29	4.58	Metacentric
14.	0.56	1.34	1.90	36.68	2.39	29.47	4.33	Submetacentric
15.	0.58	1.18	1.76	43.24	2.04	32.95	4.01	Submetacentric
16.	0.60	0.89	1.49	36.61	1.48	40.27	3.39	Metacentric
17.	0.28	1.16	1.44	35.38	4.14	19.44	3.28	Subtelocentric
X	1.98	2.09	4.07	100	1.05	48.65	9.27	Metacentric

Total Mean Haploid Length=43.89 μm
 Nombre Fondamnetale=72
 Total mean Diploid Length=87.78 μm
 Sex Mechanism =Xyp(♂)

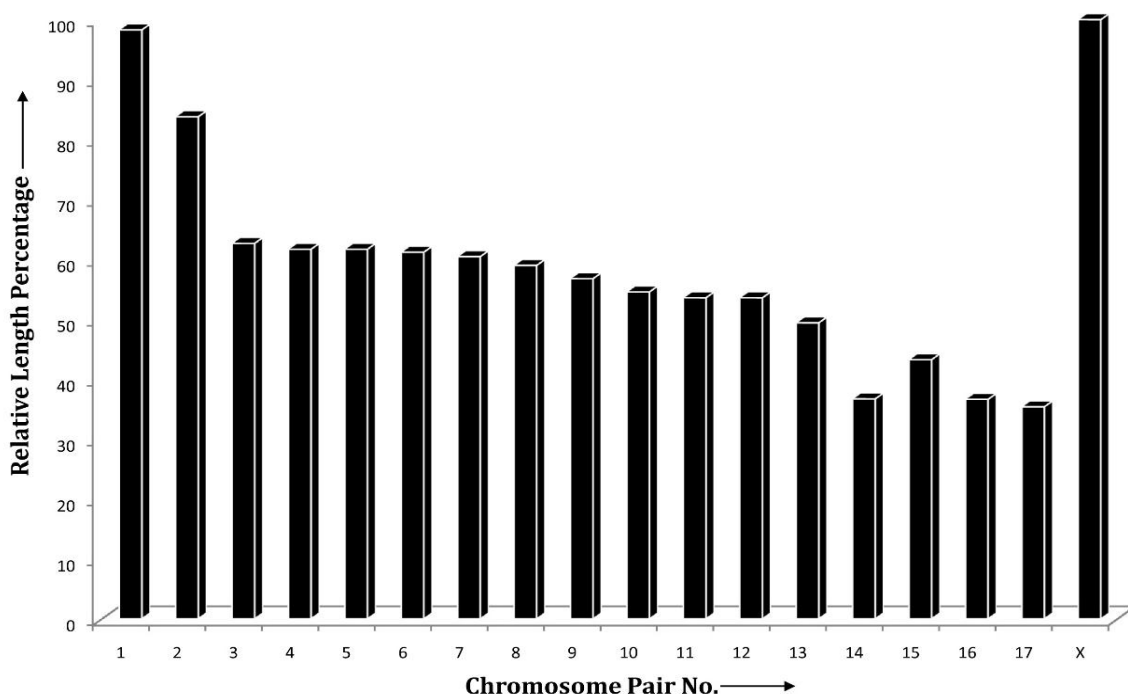


Fig. 26 Histogram of male *Hydroporus pubescens*.

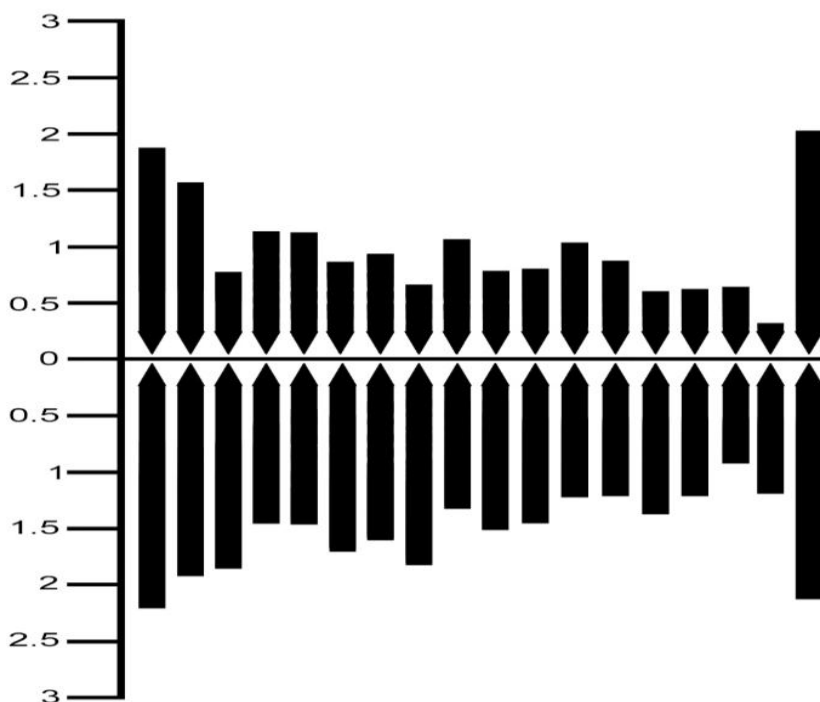


Fig. 27 An idiogram of male *Hydroporus pubescens*.

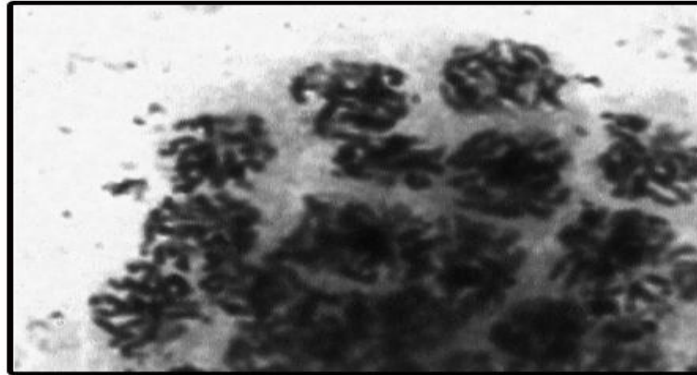


Fig. 28 Leptotene

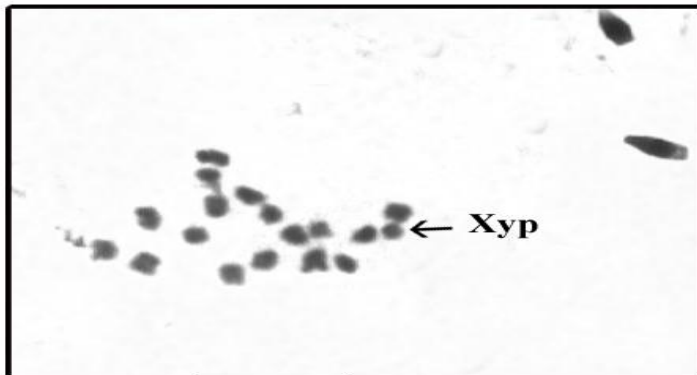


Fig. 29 Metphase - I



Fig. 30 Anaphase - I

(b) Meiotic details:

- (i) Leptotene:** A large number of cells at leptotene were observed. The chromosomes were forming a chromatin network. (Fig.28)
- (ii) Metaphase-I:** 18 bivalents (17 autosomal and 1 sex bivalents) were observed. The autosomal bivalents were cross shaped, rhomboidal and rod shaped. The sex bivalents (X0) was observed like a parachute. (Fig.29)
- (iii) Anaphase-I:** A cell at anaphase -1 was observed having 17 chromosomes at one pole and 18 chromosomes to the other indicating first meiosis to be reductional for both autosomes and sex chromosomes. (Fig.30)

Discussion:

The detailed characterization of chromosomes includes the study of whole group of characteristics that allow the identification of a particular set of the karyotype i.e. relative size, the number, length of arms, position of centromere, secondary constrictions and satellites. Since spontaneous breaking and recombination of the chromosomes originates inversions, translocations and other phenomena, we can say that the karyological differences among related species have appeared through a gradual series of modifications that altered the visible appearance of chromosomes.

FAMILY HYDROPHILIDAE

Among the Coleoptera, the family Hydrophilidae is known for being the largest in the superfamily Hydrophiloidea, comprising about 3,340 described species (Hansen, 1999; Short and Fikcek, 2011). Their representatives usually live in an aquatic environment, but there are species that inhabit leaf litter and other forms of decomposing organic matter (Fikcek *et al.*, 2010). The largest aquatic Polyphaga are allocated to the family Hydrophilidae, tribe Hydrophilini, with 198 described species. This tribe is also composed of small-sized species (less than 1 mm) and middle-sized species, among which is the genus *Tropisternus* (Short, 2010; Short and Fikcek

2011). There are few cytogenetic studies specifically for the family Hydrophilidae and most of them refer to the counting of the diploid number and identification of the sex chromosome system (Smith, 1953, Angus 1982, 1988, Shaarawi and Angus 1991). These studies reveal a frequent karyotype $2n=16+XY$ for the family. However, some variations are observed, such as $2n=14+XY$ in *Anacaena globulus* and $2n=10+neo-XY$ in *Anacaena rufipes* (Shaarawi 1989, Shaarawi and Angus 1991). Furthermore, the occurrence of triploidy and parthenogenesis was also observed in this group (Shaarawi and Angus 1991). Although some families of Coleoptera have extensive cytogenetic studies, they are scarce in the family Hydrophilidae and lack an evolutionary trend that can be traced back to the origin of this group.

During the course of present investigations, two species of family Hydrophilidae viz. *Sternolophus rufipes* (Subfamily Hydrophilinae) and *Regimbrtia attenuata* (Subfamily Berosinae) have been analysed for their chromosome numbers, sex-mechanisms and meiotic behaviour. A diploid count of $2n=18$ ($n=8+Xyp$) was reported in *Sternolophus rufipes* (Fig.1). The diploid number of 18 chromosomes was indicated by sufficient number of cells at metaphase-I which possessed 8 autosomal bivalents arranged in the form of rings and rods; the sex bivalent, however, was parachute in configuration. The karyotype consisted of seven pair of metacentric chromosomes (pair no. 1, 2, 3, 5, 6, 7, 8) and one submetacentric pair (pair no. 4) and hetromorphic pair of sex- chromosomes (X and y), whereas X was metacentric, y was submetacentric (Table-2). These results are in conformity with the findings of Agarwal, 1960; Angus, 1982,1983,1986,1988. The diploid number ($2n=18$) and type of sex chromosome system (XX/Xyp) observed in the specimens are the same as those described for many species of Helophoridae (Angus and Toledo, 2010).

Regimbartia attenuata also showed a chromosome number of $2n=18$ with Xyp sex mechanism (Angus, 1982, 1983; Agarwal, 1960; Manna and Lahiri, 1972). The Karyotype consisted of

3 pairs of metacentric (pairs no. 2,3 and 5) and five pairs of submetacentric autosomes (pairs no. 1, 4, 5, 6, 7 and 8) and a heteromorphic pair of sex-chromosomes (X and y), whereas X was submetacentric, the y was subtelocentric (Table-3). During metaphase-I, 8 rod-shaped autosomal bivalents were formed along with the Xyp. Two types of metaphase II plates were encountered, one with X-chromosome and other with Y chromosomes in addition to 8 autosomes. Thus 9 is observed as a basic number in hydrophilidae. The structural variation is accounted due to chromosomal changes occurring independently in each geographic populations.

A perusal of literature reveals chromosomal data of 105 species belonging to 22 genera and 8 subfamilies of Hydrophilidae. Of these, 47 species possess $2n=18$ constituting 44.67% of the total cytologically investigated species (Fig.31). Hydrophilidae is characterized by the occurrence of simple male sex-chromosome mechanism. Of the 106 cytologically known species sex chromosome mechanism is known in 96. Xyp is the main sex-chromosome mechanism in 66 species and the rest possess Xy type of sex mechanism. These reports are based mainly on mitotic chromosomes and the presence of large X and minute y in all of them suggest that these are Xyp cases rather than Xy. In the

present study, Xyp type of sex-mechanism was observed in the two species studied. The diploid number observed ($2n=18$ i.e. $16+Xyp$), found in most species of Hydrophilidae, associated with the Xyp sex chromosome system as a basal character of Polyphaga, allows us to infer that the species studied occupy plesiomorphic position in the chromosomal differentiation of the family. If we consider that the proposed chromosome number for the basal suborder Polyphaga is $2n=20$ (Smith and Virkki 1978), we can suggest that the karyotype of presently studied species probably arose from chromosomal fusion events that occurred in an ancestral karyotype, resulting in the formation of a karyotype with 18 chromosomes. Nevertheless, as some Hydrophilidae species have diploid number below 18. It is likely that other evolutionary events related to the reduction of the chromosome number may have occurred in the karyotype evolution of the family, placing the genus in a plesiomorphic position within it.

Thus basic Coleopteran number could not be obtained in the family Hydrophilidae. Its variable diploid numbers from 18 to 30 within 10 species only, mostly with Xyp mechanism, are suggestive of the fact that further knowledge of the family may reveal 20 as the basic number.

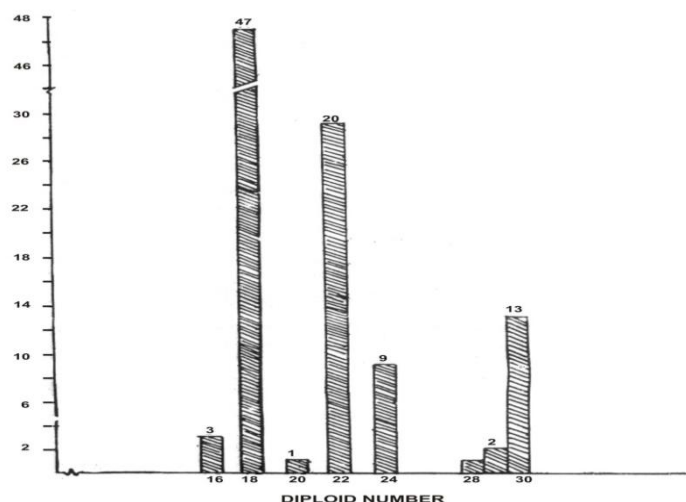


Fig. 31 Histogram showing distribution of diploid number of chromosomes in the cytologically known Hydrophilidae.

FAMILY DYTISCIDAE

The family Dytiscidae is highly diverse and estimated to include about 4,000 species in over 160 genera. Dytiscid larvae (predaceous diving beetles), well known for their piercing mandibles, inject proteolytic enzymes into their prey resulting in either subsequent ingestion of internal tissues or excruciating pain. Larvae dytiscids prey even on small vertebrates (fish and tadpoles). The members of the family Dytiscidae (Predacious diving beetles) with about 4000 species are rivaled only by the chironomidae as the largest family of fresh water animals. These beetles are found in a very wide range of aquatic habitats. Dytiscidae group is not extensively studied cytogenetically except some earlier works (Yadav and Karamjeet, 1980; Voinov, 1903).

Dytiscidae group is not extensively studied cytogenetically except some earlier works (Yadav and Karamjeet, 1980, Voinov, 1903). During the course of present investigations, two species of Family Dytiscidae viz. *Dytiscus marginalis* (Subfamily Dytiscinae) and *Hydroporus pubescens* (Subfamily Hydroporinae) have been analysed for their chromosome numbers, sex-mechanisms and meiotic behaviour. The 2n number was reported to be 38 (n=18+XY) in *Dytiscus marginalis* which is in conformity with the findings of Giardina (1901), Debaisieux (1909), Gunthert (1910), Henderson (1907), Schafer (1907), Smith and Virkki (1978), Azuma (2002). The karyotype consisted of 38 chromosomes comprising 18 autosomal pairs (7 metacentric pairs, 9 submetacentric pairs and 2 pairs of subtelocentric pairs X was observed to be submetacentric and Y to be subtelocentric (Table- 4). In the *Hydroporus pubescens* (Subfamily Hydroporinae), the 2n number was reported to be 34 (17 pairs of autosomes and sex-chromosomes which are XO in male and XX in female. The autosomes were mainly metacentric or nearly so. The rest of the autosomes show fairly gradual decrease in size. X chromosome is metacentric and clearly larger than the last chromosomal pair. These observations are in conformity with the findings of Yadav and Karamjeet (1981a, b, and c); Ahmed *et al.*, (2000). Balke and

Fery (1993) reported that *Hydroporus pubescens* is closely related to *Hydroporus humilis*. However, there are no published data on *Hydroporus* chromosomes but Angus (1989) reported 20 pairs of chromosomes plus XO/XX in other species of Hydroporinae.

When Smith and Virkki (1978) compiled their list of beetles whose chromosome numbers were known, they gave data for 2120 species, including 138 named species belonging to the suborder Adephaga. Of these 21 were Dytiscidae and 7 Gyrinidae. By 1984, the number of dytiscid species whose chromosome numbers were known had increased to 32 though five of these were unidentified (Yadav *et al.*, 1984). Interestingly, the total number of world species of Dytiscidae is about 4080 (Nilsson-Örtmann and Nilsson 2010). Numbers for Dytiscidae have also continued to increase. Ahmed *et al.*, (2000) gave data on 1 species of *Hydrovatus* Motschulsky, 1 *Hydroporus* Clairville and 3 *Nebrioporus* Régimbart (Hydroporinae), 1 *Agabus* Leach (Agabinae), 1 *Colymbetes* Clairville (Colymbetinae) and 1 *Eretes* Laporte and 1 *Hydaticus* Leach (Dytiscinae). Aradottir and Angus (2004) gave information on 7 species of *Ilybius* Erichson (Agabinae), Dutton and Angus (2007) described the karyotypes of 7 species of the "*Stictotarsus griseostriatus* (De Geer) group" (now in the genus *Boreonectes* (Hydroporinae). Tatton and Angus (2011) reported on 30 species related to *Deronectes* Sharp (Hydroporinae), of which 27 had no previously published data, bringing to total number of dytiscid species with known chromosome numbers to about 100 (Angus, 2011). The data has been gathered over more than 25 years.

A perusal of literature reveals that only 32 species of the family Dytiscidae are cytologically known (Fig.32). Yadav and Karamjeet (1981a) found a higher range of chromosome numbers (2n=40 to 45) in seven species of Indian Dytiscids. Dytiscids possess simple sex-chromosome mechanisms (Yadav *et al.*, 1984) with predominant XO formula in male (Smith and Virkki, 1978) which frequently gives rise to species with neo- XY (White, 1973). *Dytiscus*

marginalis showed XY male sex chromosome mechanism while XO was reported in *Hydroporus pubescens* in the present study. The variations observed in the structure of chromosomes may be due to pericentric inversions occurring independently in each population. Dytiscidae possess simple sex-chromosome mechanism, X0 chromosome in male individuals (Ahmed *et al.*, 2000). Such system was also reported earlier in other Dytiscids species like *A. confinis* and *Rantus notatus* (Smith, 1953 and Suortti, 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). Actually no clear picture with regard to a primitive Dytiscid karyotype and the direction it might have taken during the course of evolution emerges from the available data. However, the number of chromosomes, metacentric nature of most of the elements and their large size confirm to $9AA+Xyp$ as the basic formula for the Coleoptera (Smith, 1950). The variable diploid numbers from 20 to 46 have been recorded in the Dytiscidae. More data is needed before any conclusion can be reached. Inspite of many taxonomic studies on aquatic beetles, there has been fewer attempts to explore the karyology of aquatic beetles.

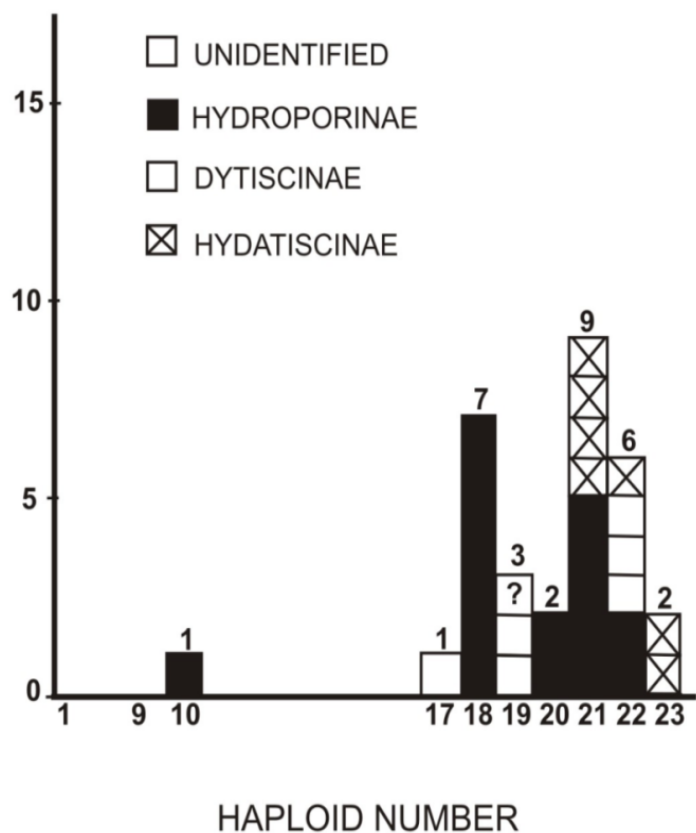


Fig. 32. Histogram showing distribution of Haploid number of chromosomes in the cytologically known Dytiscidae

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References:

- Shantibala, T., Lokeshwari, R.K., Gourshyam, Th. and Somkuwar, B.G. (2012) Meiman:Database exploring Medicinal and Edible insects of Manipur. *Bioinformation*, 8(10), 489-491.
- Benazzi, M. (1973) Cyto-taxonomy and Evolution:General remarks. In *Cytotaxonomy and Vertebrate Evolution*, 3-14.
- White, M.J.D. (1963) Principles of karyotype evolution in animals. *Genetics Today*, 1:391-397.
- Agarwal, U. (1960a) Chromosome number and sex-mechanism in sixteen species of Indian Coleoptera. *Curr. Sci.*, 29:40.
- Agarwal, U. (1960b) Chromosome complement and meiosis in two species of Bruchidae (Coleoptera). *Proc. Nat. Acad. Sci. (India) B.IV.*, 30:353-356.
- Angus, R. B. (1982) Separation of two species standing as *Helophorus aquaticus* (L.) (Coleoptera, Hydrophilidae) by banded chromosome analysis. *Syst. Entomol.*, 7:265-281.
- Angus, R.B. (1983) Separation of *Helophorus grandis*, *maritimus* and *occidentalis* sp. N. (Coleoptera:Hydrophilidae) by banded Chromosome Analysis. *Syst. Ent.*, 8:1-13.
- Angus, R.B. (1986) Revision of the Palaearctic species of the *Helophorus minutus* group (Coleoptera:Hydrophilidae), with chromosome analysis and hybridization experiments. *Syst. Ent.*, 11:133-163.
- Manna, G.K. and Lahiri, M. (1972) Chromosome complement and meiosis in forty-six species of Coleoptera. *Chromosome information service*, 13:9-11.
- Shaarawi, F.A. (1989) Chromosomal investigation on European Hydrophilidae (Coleoptera). Ph. D. Thesis, University of London, p.176.
- Yadav, J.S, and Dange, M.P. (1991) Chromosome studies on six species of Indian water beetles (Hydrophilidae:Coleoptera). *Research bulletin of the Punjab University*, 42:61-71.
- Yadav, J.S., Karamjeet, K. and Yadav, A.S. (1984) Karyological investigations on seven species of Dytiscidae (Adephaga:Coleoptera). *Turk Btkikor Derg.*, 8:3-13.
- Yadav, J.S. and Karamjeet, K. (1980) Chromosome number and sex determining mechanism in thirty species of Caraboidea (Adephaga:Coleoptera). *Cordulia*, 6:20-23.
- Voinov, D.B. (1903) La spermatogenese d'ete chez le *Cybister roeselli*. *Archives de Zoologie Experimentale Et Generale*, 4:173-260.
- Giardina, A. (1901) Origine dell'oocite e delle cellule nutritive nel *Dytiscus*. *Int. Monastsschr. Anat. Physiol.* 18:417-484.
- Debaisieux, P. (1909) Les debuts de loogenese dans le *Dytiscus marginalis*. *cellule*, 25:205-240.
- Gunther, T. (1910) Die Eilbildung der Dytisciden. *Zool. Jahrb.*, 30:301-372.
- Azuma, A., Yoshitaka, O. and Koji, K. (2002) Chromosomes of three species of Dytiscids (Coleoptera, Dytiscidae). *Japanese Journal of Entomology*, 5(4), 145-154.
- Hansen, M. (1999) World Catalogue of Insects. Volume 2. Hydrophiloidea (s.str.) (Coleoptera). Apollo Books, Stenstrup. p.416.
- Short, A.E.Z. and Fikacek, M. (2011) World catalogue of the Hydrophiloidea (Coleoptera): additions and corrections II(2006 2010). *Acta Entomol. Mus. Natl. Pragae*, 51:83-122.
- Fikáček, M., Gentili, E. and Short, A.E.Z. (2010) Order Coleoptera, family Hydrophilidae. *Arthropod fauna of the UAE.*, 3:135-165.
- Short, A.E.Z. (2010) Phylogeny, evolution and classification of the giant water scavenger beetles (Coleoptera:Hydrophilidae. Hydrophilini. Hydrophilina). *System. Biodivers*, 8:17-37.
- Short, A.E.Z. and Fikacek, M. (2011) World catalogue of the Hydrophiloidea (Coleoptera):additions and corrections II(2006 2010) *Acta Entomol. Mus. Natl. Pragae*, 51:83-122.
- Smith, S.G. (1953). Chromosome numbers of Coleoptera. *Heredity*, 7:31-48.

- Angus, R. B. (1988) A new sibling species of *Helophorus* F. (Coleoptera:Hydrophilidae), revealed by chromosome analysis and hybridisation experiments. *Aquat. Insects.*, 10:171-183.
- Shaarawi, F.A. and Angus, R.B. (1991a) A chromosomal investigation of five European species of *Anacaena* Thomson (Coleoptera:Hydrophilidae). *Entomologica Scandinavica*, 21, 415-426.
- Shaarawi, F.A. and Angus, R.B. (1991b) Chromosomal analysis of some European species of the genus *Berosus* Leach (Coleoptera:Hydrophilidae). *Koleopterologische Rundschau*, 61:105-110.
- Angus, R. B. and Toledo, M. A. (2010) A new, chromosomally distinct Italian *Helophorus* F. similar to *H. aequalis* Thomson, and notes on some other species in Italy (Coleoptera:Helophoridae). *Koleopterol. Rundsch.*, 80:103-111.
- Smith, S.G. and Virkki, N. (1978) List of chromosomes numbers of Coleoptera, In: B. John (ed.); *Animal Cytogenetics*, Vol. 3, Insecta 5. Gebrüder Borntraeger, Berlin -stuttgart, p.366.
- Schaefer, F., (1907) Spermatogenese von Dytiscidae (Ein Beitrag zur frage der chromatin reduction). *Zool. Jahrb.*, 23: 535-586.
- Yadav, J.S. and Karamjeet, K. (1981a) Chromosome studies on three species of Cicindelidae (Adephaga:Coleoptera) from Haryana. *Zool.Anz.Jena.*, 206:121-128.
- Yadav, J.S. and Karamjeet, K. (1981b) Chromosome studies on five species of Carabidae (Adephaga:Coleoptera) from Haryana. *Proc.2nd Indian Symp.Exp.Zool.*, Baroda (Abstr.), 67-68.
- Yadav, J.S. and Karamjeet, K. (1981c). Chromosome studies on seven species of Dytiscidae (Adephaga:Coleoptera) from Haryana. *Proc.4th Indian Cong.Cytol. Genet.* Bhagalpur (Abstr.), 9
- Ahmed, R.S., Angus, R.B., Zalat, S. and Shaarawi, F. (2000). Chromosomal analysis of some Egyptian diving beetles (Coleoptera:Dytiscidae). *Egyptian Journal of biology*, 2:76-84.
- Balke, M. and Fery, M. (1993) Taxonomic notes on Western palaearctic species of *Hydroporus* clairville and *coelambus* Thomson (Coleoptera:Dytiscidae) *Ann. Soc. Entomol. Fr. (N.S.)*, 29(1), 89-101.
- Angus, R.B. (1989). Towards an atlas of *Helophorus* chromosomes. *Newsl.Balfour-Browne Club*, 44:13-22.
- Nilsson-Ortmann, V. and Nilsson, A.N. (2010) Using taxonomic revision data to estimate the global species richness and characteristics of undescribed species of diving beetles (Coleoptera:Dytiscidae). *Biodiversity Informatics*, 7(1):1-16.
- Aradottir, G.I. and Angus, R.B. (2004) A chromosomal analysis of some water beetle species recently transferred from *Agabus* Leach to *Ilybius* Erichson, with particular reference to the variation in chromosome number shown by *I. montanus* Stephens (Coleoptera:Dytiscidae). *Hereditas*, 140:185-192.
- Dutton, L.A. and Angus, R.B. (2007) A karyosystematic investigation of a group of sibling species related to *Stictotarsus griseostriatus* (De Geer) (Coleoptera:Dytiscidae). *Comparative Cytogenetics*, 1:3-16.
- Tatton, A.G and Angus, R.B. (2011) A karyosystematic analysis of some water beetles related to *Deronectes* Sharp (Coleoptera, Dytiscidae). *Comparative Cytogenetics*, 5(3):173-190.
- Angus, R.B. (2008) Further karyosystematic investigation of the *Stictotarsus griseostriatus* (De Geer) group of sibling species (Coleoptera:Dytiscidae). *Comparative Cytogenetics*, 2:151-156.
- Angus, R. B.(2010) A third karyosystematic investigation of the *Stictotarsus griseostriatus* (De Geer) group of sibling species (Coleoptera:Dytiscidae). *Comparative Cytogenetics*, 4(1):13-20.
- Angus, R.B., Clery, M.J., Carter, J.C., and Wenczek, D.E. (2013) Karyotypes of some medium-sized Dytiscidae (Agabinae and Colymbetinae) (Coleoptera). *Comparative cytogenetics*, 7:171-190.
- White, M.J.D. (1973). *Animal cytology and Evolution*, 3rd Edition Cambridge University Press, London.

Suortti, M. (1971) Spermatogenesis of some species of Dytiscidae (Coleoptera). *Annales Zoologici Fennici*, 8:390-393.

Abdel-Haleem, A.A., Sharaf, H.M. and 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)*, 2, 163-166.

Smith, S.G. (1950) The cytotaxonomy of Coleoptera. *Can. Entom.*, 82:58-68.