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Meiotic chromosomes of *Sternolophus rufipes* (Coleoptera, Hydrophilidae)

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Abstract
The cytotaxonomy of the hydrophilid, *Sternolophus rufipes* Fabricius 1792 was examined in this study using meiotic cell division from testis. The hydrophilini subtribe hydrophilina is composed of around 200 described species, including all known water scavenger beetles. The tribe hydrophilini is the most recognizable group in the family due to its large size and presence of sternal keel. In this study, we analysed 8 specimens of *Sternolophus rufipes* from the different wetland of Manipur. Chromosome analyses showed 2n= 18 with a sex chromosome system of the Xyp type ($2n=18=16+Xy_p$). All the autosomes, including the X chromosome were metacentric and y_p. The genus *Sternolophus* currently has nine valid species worldwide. In India only *Sternolophus rufipes* had been reported so far. In future exclusive exploration of this genus through morphometrics, cytotaxonomy and molecular studies are much for thorough diversity assessment in Manipur. In meiotic studies of *Sternolophus rufipes*, flame drying methods gives much better results than Acetocarmine staining. The diploid count was 18 with $16+Xy_p$ karyotype. The Y chromosomes were dots. The meiotic stages could be visualized in 100X due to smaller size of the chromosomes. There were not much deviation on the general features of the meiotic stages except that the diplotene stages were somewhat deviate from the general configuration in the sense that bivalents were extremely condensed and most of them were rounded in shape. The chiasmatic like features could be observed in nearly equatorially align diakinesis. Much is needed to be studied with this species in diversity and cytology in the future.

Keywords: *Sternolophus*, male, meiotic chromosomes, chromocenters, scavengers

1. Introduction
Coleoptera popularly known as beetles represents 25% of all described species of animal and plants with approximately 358, 000 described species all over the world. They are herbivores, fungivores or carnivores, scavenger, predators in the larval and adult stages [1]. A total of thirty-four genera of aquatic Hydrophilidae is known from the Palearctic and Oriental regions. Twenty-six of them have been recorded from China, where the borders of these two geographical realms overlap. Most hydrophilid genera are not confined strictly to one of these realms [2].

Among the Coleoptera, the family Hydrophilidae is known for being the largest in the super-family Hydrophiloidea, comprising about 3,340 described species [3, 4]. Their representatives usually live in an aquatic environment, but there are species that in- habit leaf litter and other forms of decomposing organic matter [5]. 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* Solier 1834 [4, 6]. Given that Coleoptera is of great ecological significance coleopters are widely used in several studies, including those that evaluate the ecology, morphophysiology, genetic structure, and phylogeny of the order [6, 7, 8, 9]. 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 [10, 11, 12, 13].

The genus *Sternolophus* Solier, 1834 is widely distributed in the tropics of the Old World, with only few species occurring in the temperate zones. In a recent taxonomic revision of the genus by Nasserzadeh and Komarek [14] the number of species was increased from nine (Hansen 1999) to 17. So far the diploid count of *Sternolophus rufipes* was 18 in both male and female individuals [15].

Although some families of Coleoptera have many 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. In this paper, we describe the cytogenetic analysis of *Sternolophus rufipes* particularly the meiotic chromosomes and report unprecedented cytogenetic data on the specie. In addition, the chromosomal organization and the possible events involved in the karyotype evolution of the family are discussed.

2. Materials and Methods

50 (fifty) alive specimens of *Sternolophus rufipes* were collected from different aquatic habitats like pond and lakes with help of net from 31st May 2015 to 21st November, 2017. The specimens were brought to the laboratory and identified according with available reference books particularly males were identified. The males were dissected to take out the testes.

2.1 Taxonomy background of the insect

Diagnosis: Body elongated oval, moderately convex, 12.99 mm long and 5.96 mm wide. Antennae nine segmented with three segmented club. Prosternum deeply cleft posteromedially, carina with small tuft of setae anteriorly. Mesosternal keel with small notch set with setae anteriorly. Posterior spine of metaventrite short to long extending upto the second abdominal segment. Elytra without sutural stria, meso and metatibial spurswithut row of spicules, hind femora pubescent at extreme basally.

Distribution

India: Manipur (Bishnupur).

Elsewhere

California, South America, North America.

Remark

Elytra without punctures, elevated portion of meso and meta sternum continuous, forming a common sterna keel that is produced backward into a long horizontal spines.

2.2 Meiotic Chromosome preparation

2.2.1 Squashed preparation: The testis were dissected out and put in fixative (Carnoy's fluid I) comprising of 1 part glacial acetic acid and 3 parts of ethanol by v/v for 24 hours. The seminiferous tubule were stained with 2% acetocarmine for 30 minute and softened with 45% glacial acetic acid. The tubules were covered with coverslide and finally applied thump pressure by folding in between the blotting paper.

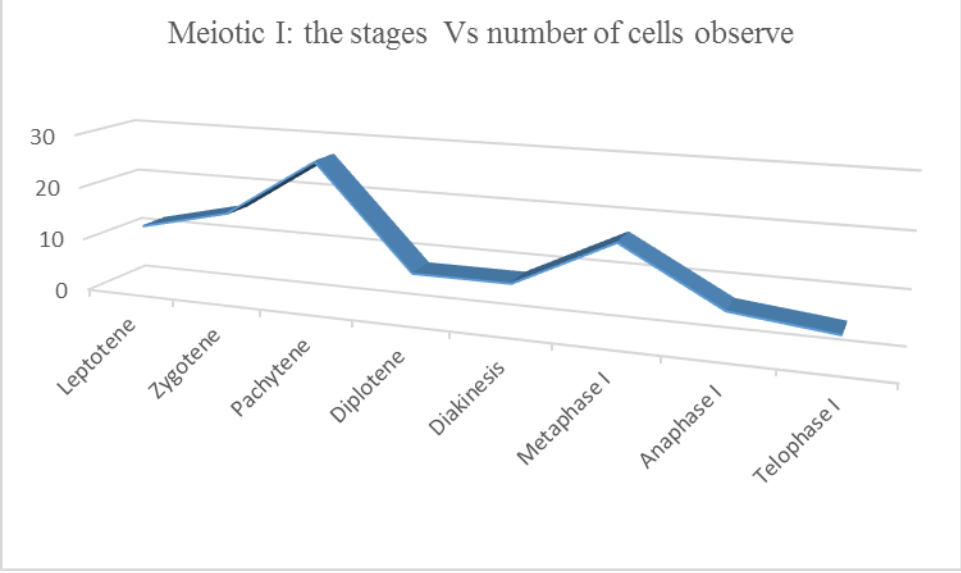
2.2.2 Flame drying method: The chromosomes were prepared according to Banerjee [16] with slight modifications. The testis were dissected out in KCl (0.56M) and yellowish fat and other unwanted materials were taken out. This makes the exposure the cells to KCl atleast for 20 minutes. Then the cells were treated to 45% Glacial acetic acid for 5 minutes and make slight crushing with micro pestle in the micro centrifuge tube and aspirated five times. The cell suspension was centrifuged at 5000 rpm for 10 minutes. The supernatant was discarded and pellet was carefully aspirated with a pipette so as to avoid clumping with fresh fixative and keep for 10 minute at room temperature. The centrifuge again at 5000 rpm for 10 minutes and pellet was re-suspended with fresh fixative and spread on pre-chilled slides soaked with methanol. The slide was burned for 20 seconds then stored in a desiccator for three days before staining to observe the Meiosis.

2.2.3 Staining: The slides were stained in 2% Giemsa stained for 20 minutes and let dry. After staining the slides were dried at room temperature and made permanent with DPX with 22X60 cover slide and dried till the DPX was properly dry.
The slides were then mounted on the microscope and inspects stages of meiosis. The selected stages were taken snaps at 100X with Coolpix digital camera attached to the microscope. In total 20 cells of each cell stage were taken into account and clear stages were taken photographs.

2.3 Statistical analysis: The number of meiotic I cells were randomly observed and each cells were counted. The comparative number of each sub stages plotted on a graph to show the tentative status of the cell cycle.

Table 1: Comparative Meiotic I: the stages Vs number of cells observed in randomly selected observations

Cell Stages	Lepto	Zygo	Pachy	Diplo	Diakine	Meta I	Anap I	Telo I
No. of cells	12	16	27	8	8	17	7	5



3. Results and Discussion

The specimen in the present study is confirmed to be *Sternolophus rufipes* (fig. 1 A, B) The aedeagus is the special feature for identifying the male (fig.1 C, D) and testes were somewhat round (fig. 1 E) in shape with 85 numbers of seminiferous tubules in each testis. Each seminiferous tubules measure around one millimeter (1 ml) (fig. 1 E) and medium size sperms were about 14.23 micron in length. The lack of an elytral sutural stria, the basal pubescence of the hind femora and unemarginated prosternal carina will separate *Sternolophus* from other Hydrophilina. The smaller size will distinguish members of the genus from nearly all other Old World taxa in the subtribe. Its distribution is largely restricted to the Old World tropics, including Africa, Central and Southeast Asia, and Australasia [6]. Toussaint *et al*. [17] postulated an Afro-tropical origin for *Sternolophus*, dispersing toward Australia in the Oligocene/Miocene. There are many New Cenozoic fossil findings of taxa closely related to *Sternolophus* in Europe and North America [18, 19, 20], whereas the only record of this genus is a dubious fossil likely belonging to *S. rufipes* from the Early Pliocene of the Tsubusagawa Formation in Japan [21]. The current distribution of *Sternolophus* in the Old World, i.e. without protruding into northern Asia, Europe, Tasmania and New Zealand [14], which were largely covered by ice, and its absence in the fossil records from Europe and America, suggest a sensitivity of this group to climate change and glacial periods as inhibitor factors for its distribution, and also highlight the effect of eustatic changes in accelerating its dispersal in the Old World towards Australia.

The meiotic stages from seminiferous cells were studied through flame drying methods (fig. 2) and Acetocarmine (fig.3). The comparatively the flame drying method was far better than the Acetocarmine staining because the flame drying method gave well spread meiotic stage which is five times than in the Acetocarmine squashed method. The reason behind this might be exposure to hypotonic solution which gives much swimming areas for the chromosomes. Overall quality of the cells in the present study were somewhat not upto the mark-blurred as compare to the typical grasshopper meiotic stages. These might be due to much smaller chromosomes in this species and less exposure to hypotonic solution as well as to fixative solution. So in future studies increase the exposure timing to hypotonic solution as well as to fixative solution.

Analyses of spermatogonial metaphases of *Sternolophus rufipes* showed 2n=18 with 8+Xy_p configuration as reported [15]. The analyses of male meiotic cells revealed a sex chromosome system of the Xy_p type (fig. 2 L, Q). All the autosomes were metacentric, including the X chromosome. The autosomal pairs gradually decreased in size. The y_p chromosome was a dot-like structure (fig. 2 L). Leptotene configuration of bouquet arrangement was roughly seen as heteropicnotic (blocks in fig. 2A in right side) and fairly lightly stained chromatins (in left side). The pairing of the homologous chromosomes could be visualized with shorter chromosome length in zygotene stage. The pachytene stage with thickened and shorter chromosomes with distinct 9 in numbers were maximum in random observations (Table 1 and fig. 4). Diplotene stages were somewhat deviate from the general configuration in the sense that bivalents were extremely condensed and most of them were rounded in shaped. The chiasmatic like feature could be observed in nearly equatorially align Diakinesis (fig. 2 E). Rest of the stages of Meiotic I were generally conventional. The dots like features of y_p could be observed in fig. 2 l and Q.

The diploid number (2n=18) and type of sex chromosome system (XX/Xyp) observed in specimens of *Sternolophus* are the same as those described for many species of coleopters, such as Helophoridae [22], Melyridae [23, 24], and Scarabaeidae [25] proposed that 2n=20 with meioformula 9+Xyp is the ancestral karyotype (basal) in the order and that some families are more conserved [26, 27, 28]. However, several cytogenetic studies show a great variability with respect to both chromosome number and types of sex chromosome systems among different families, in which one can observe a variation from 2n=4 in *Chalcolepidius zonatus*, Family Elateridae [29] to 2n=69 in Ditomus capito, Family Carabidae [30].

The beauty of the spermatogonal cells in this species was the presence of huge numbers of chromocenters numbering 24 and 33 (fig. 2 N and O respectively). These were the constitutive heterochromatin present in the genome and could be visualized with fluorescents [31], or Giemsa [32] or chromosome banding techniques [33]. The different in numbers and size of the chromocenters inside the cells could be explained with the fusion and disintegration of these structures [34] into their respective centromeres or other chromosome components. Till date no reference work on the meiotic division of this species could be found, so a lot of discovery will be needed on this species in future studies.

The figure (fig 4) showed that the cells observed randomly were having maximum in Pachytene and Metaphase I stages while other stages were moderate. From this we could conclude that the cells were rapidly dividing preparing for the next generation.

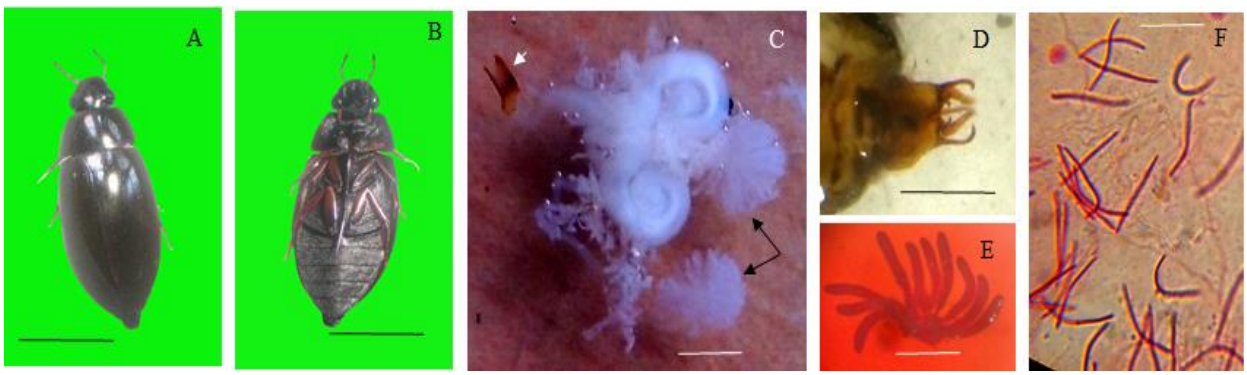


Fig 1: The adult and parts of aedeagus of *Sternolophus rufipes.* A and B The adult dorsal view of *Sternolophus rufipes*, C) the dissected male organs of the specimen, black arrows point the testes and white arrow points the aedeagus and D) magnified view of aedeagus, E) Seminiferous tubules stained with Acetocarmine each individual cell measures 1 millimeter, F) Sperm cells each measure 14.23 microns. Bar represents 5 mm in A, B; others 0.5 ml.

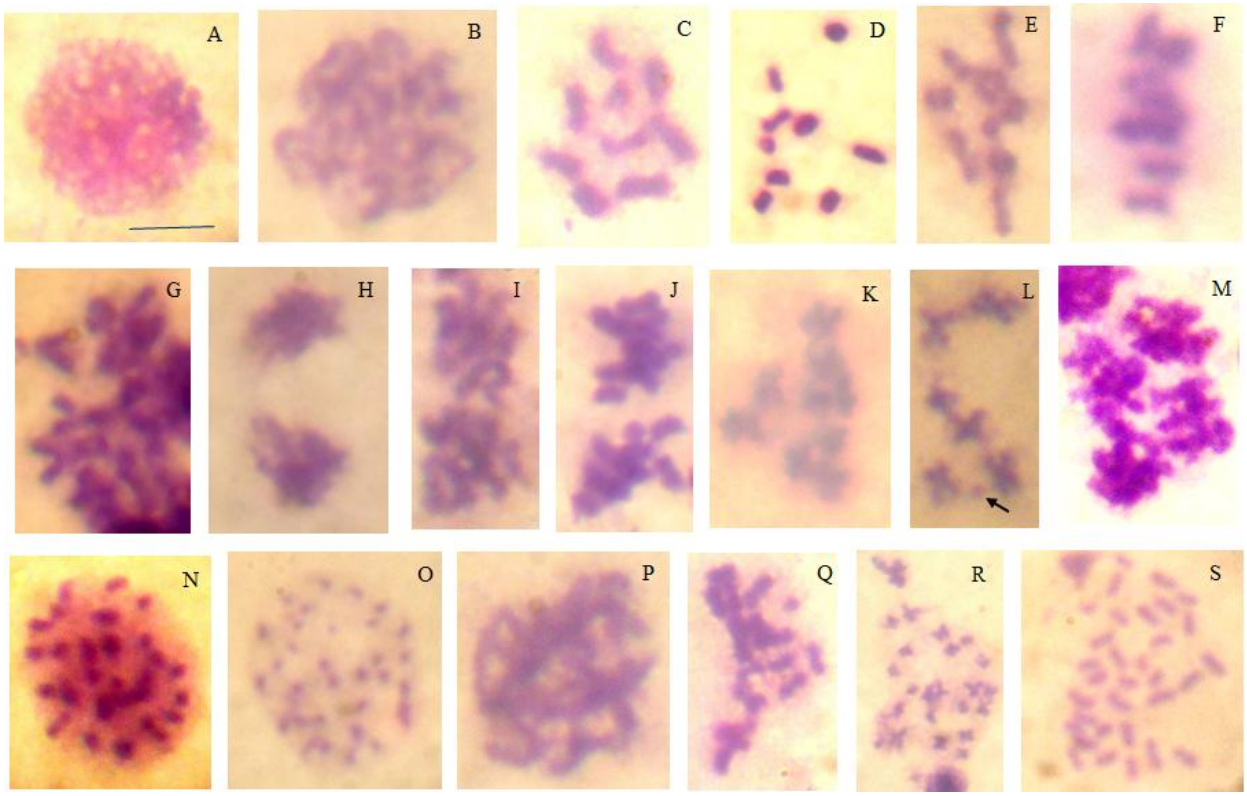


Fig 2: The different stages of meiotic cell division from testes of *Sternolophus rufipes*. Leptotene A), Zygotene B), Pachytene C), Diplotene D), Diakinesis E), Metaphase I F), Anaphase G), Teolphase I H), Prophase II I), Metaphase II J), Anaphase II K), Anaphase II with Y chromosome (black arrow, L), Telophase II M). The spermatogonal mitosis interphase with fused chromocenters N), with scattered chromocenters O), Prophase P), Metaphase Q), Anaphase R) and Telophase S). Bar represents 5µm.

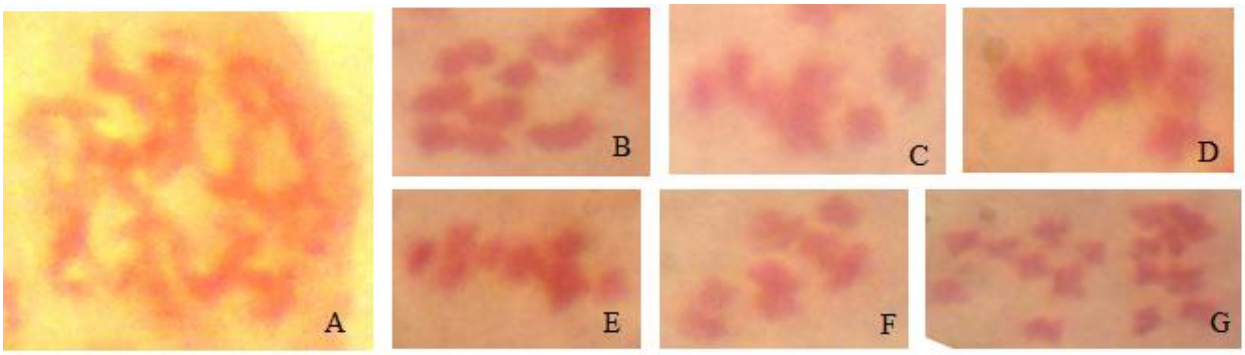


Fig 3: The different stages of meiotic cell division from testis of *Sternolophus rufipes* prepared with Squashed method stained with Acetocarmine. Zygotene A), Pachytene B), Diplotene C), Diakinesis D), Metaphase I E), Anaphase II F), Spermatogonal anaphase G). Bar represents 5µm.

4. Conclusion

In meiotic studies of *Sternolophus rufipes*, flame drying methods gives much better results than Acetocarmine staining. The diploid count was 18 with $16+Xy_p$ karyotype. The Y chromosomes were dots. The meiotic stages could be visualized only in 100X due to the smaller size of the chromosomes. There were not much deviation on the general features of the meiotic stages except that the diplotene of Meiosis I stages were somewhat deviate from the general configuration in the sense that bivalents were extremely condensed and most of them were rounded in shaped. The chiasmatic like feature could be observed in nearly equatorially align diakinesis. Much is needed to be studied with this species in diversity and cytology in the future.

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