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# Linear characterization of chromosomes in two species of family Euphaeidae (Odonata: Zygoptera)

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**Abstract**
Chromosome complement and meiotic behavior of chromosomes in two species of family Euphaeidae have been described by conventional staining, C- banding, silver nitrate staining and sequence specific staining. Anisopleura *lestoides* reveals two complements n=12 (without m chromosomes) and n=13 (with m chromosome), while *Bayadera indica* possess n=13m. In *Anisopleura lestoides* all the autosomal bivalents including m bivalent are showing terminal C-bands and NOR bands. Whereas in *Bayadera indica*, terminal C-bands on both ends in 9 autosomal bivalents and C-bands on one side in 3 bivalents, whereas terminal NOR bands are present in 10 autosomal bivalents, 1 NOR band on one side in 1 bivalent, m bivalent is C-negative and NOR-negative. X chromosome is C-positive and NOR rich in both the species. In the sequence specific staining, two autosomal bivalents show both $CMA_3$ and DAPI bright signals and remaining bivalents including m bivalent and X chromosome possess $CMA_3$ bright and DAPI dull regions in *Anisopleura lestoides*, while all the chromosomes show both DAPI and $CMA_3$ bright signals in *Bayadera indica.*

**Keywords:** odonata, Euphaeidae, C-banding, Silver nitrate staining, Sequence specific staining

## Introduction
Family Euphaeidae belongs to superfamily Calopterygoidea of suborder Zygoptera. Family includes 68 species of 12 genera worldwide, while 18 species under 6 genera are found in India. So far, 3 species have been cytologically studied, among these, 2 species have also been reported from India (Chatterjee and Kiauta, 1973 [2, 5]; Kiauta, 1975 [6], Kiauta and Kiauta, 1976 [7]; Goni and Abenanta, 1982; Tyagi 1982) [11]. During the present study, chromosome complements of *Anisopleura lestoides* and *Bayadera indica* of family Euphaeidae have been linearly characterized by conventional staining, C-banding, silver nitrate staining and sequence specific staining. Both the species possess n=13m as the chromosome number, while *Anisopleura lestoides* also show n=12 (without m chromosomes) complement.

## Materials and methods
Live male specimens were collected from running water bodies present in Himachal Pradesh, India, during post-monsoon seasons of year 2015. Specimens were dissected in 0.67% saline solution to remove the testes and were fixed in freshly prepared Carney's fixative (3:1, absolute alcohol: glacial acetic acid) for the chromosomal preparations. Cytological studies like conventional staining (Carr & Walker, 1961) [1]; C-banding (Sumner, 1972) [9]; silver nitrate staining (Howell & Black, 1980) [4] and sequence specific staining (Rebagliati *et al.* 2003) [8]. Have been performed on air dried slides. Relevant meiotic plates have been photomicrographed.

## Results
### Conventional staining
In both the species, 25 elements are present in spermatogonial metaphase, which include 24 autosomes, m chromosomes and one small X chromosome (Fig. 1a, 2e). During diakinesis and metaphase-I, 13 elements are present, which differentiated as 11 autosomal bivalents, m-bivalent and X univalent (Figs. 1b, 1c, 2f, 2g). Moreover, in *Anisopleura lestoides*, n=12 (without m bivalent) has also been observed during diakinesis and metaphase-I (Fig. 1d, 1e).

### C- Banding
During diakinesis, in n=13 (Fig. 1f) and n=12 (Fig. 1g) complements, all the autosomal bivalents including m bivalent are showing terminal C-bands in *Anisopleura lestoides*, while 9 autosomal bivalents possess terminal C-bands on both sides, 3 bivalents show one terminal C-band and m bivalent is C-negative in *Bayadera indica* (Fig. 2h, 3a). X chromosome is C-positive for the entire length in both the species.

### Silver nitrate staining
During diakinesis, all the autosomal bivalents show NOR bands at chiasmatic non chiasmatic ends in *Anisopleura lestoides* (Fig. 1h), while 10 autosomal bivalents show terminal NOR bands, 1 bivalent possesses NOR band only on one side in *Bayadera indica* (Fig. 3b). M bivalent is NOR- negative and X chromosome is NOR rich in both the species.

### Sequence specific staining
During diakinesis and metaphase-I of *Anisopleura lestoides*, two autosomal bivalents show both $CMA_3$ and DAPI bright signals, whereas remaining bivalents including m bivalent and X chromosome possess $CMA_3$ bright and DAPI dull signals (2a, 2b, 2c, 2d). On the other hand, in diakinesis and anaphase-I of *Bayadera indica*, all the autosomal bivalents including m bivalent and X chromosome show both DAPI and $CMA_3$ bright signals (Figs. 3c, 3d, 3e, 3f).

## Discussion
Family Euphaeidae possesses n=13 as the type number. So far, 3 species have been studied cytologically. Among these,

*Anisopleura comes* (Kiauta and Kiauta, 1976, 1982) [7] and *Bayadera indica* (Chatterjee and Kiauta, 1973 [2]; Kiauta, 1973, 1975) [5, 7].possess the type number (n=13) of the family, while two complements n=13, 14 due to fragmentation of chromosome has been reported in *Anisopleura lestoides* (Tyagi, 1982, 1986) [10, 11]. During present study, *Bayadera indica* collected from Andretta and Kullu areas of Himachal Pradesh and possesses n=13m, which is in accordance to the earlier reports. However, *Anisopleura lestoides* possesses n=13 (with m chromosome) and n=12 (without m chromosome) complements from Andretta (Himachal Pradesh). While n=13, 14 due to autosomal fragmentation have been reported by (Tyagi, 1982, 1986) [10, 11] from Dehradun (Uttrakhand). Variation in the chromosome number in the species indicates that the chromosome complement of the species is unstable and under the process of karyotype evolution.

Linear characterization of chromosomes has not been reported on any species of family Euphaeidae. During the present study, in *Anisopleura lestoides* and *Bayadera indica*, C-bands and NOR bands are mostly present at terminal ends on one sides/both sides of the chromosomes, while m bivalent possesses chromosomes is C-negative and NOR negative in *Bayadera indica* and X chromosome is C-positive and NOR positive in both the species. Presence of C-heterochromatin on the terminal ends indicate that the centromeric activity is localized at the terminal ends during the meiosis, which helps in segregation and movement of chromosomes on the spindle. This behaviour of localization of C-heterochromatin on terminal ends is very common feature in other insect orders having holocentric chromosomes.

Sequence specific staining has been done to locate the presence of AT and GC rich regions. In *Anisopleura lestoides*, two autosomal bivalents show both $CMA_3$ and DAPI bright signals, while other autosomal bivalents possess bright $CMA_3$ and dull DAPI signals, which indicate the presence of more GC rich regions than AT rich regions. On the other hand, *Bayadera indica* reveals DAPI and $CMA_3$ bright signals, which are due to the presence of interspersed both GC and AT rich regions. Results of C-banding and silver nitrate staining correspond to the AT and GC rich regions of sequence specific staining in both the species. During the present study, karyotypie variation in the chromosome complement of *Anisopleura lestoides* has been observed and linear characterization of chromosomes have been done for the first time in both the species.

## Conclusion

During the present study, karyotypie variation in the chromosome complement of *Anisopleura lestoides* has been observed and linear characterization of chromosomes have been done for the first time in both the species.

## Acknowledgment

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## Figure legends

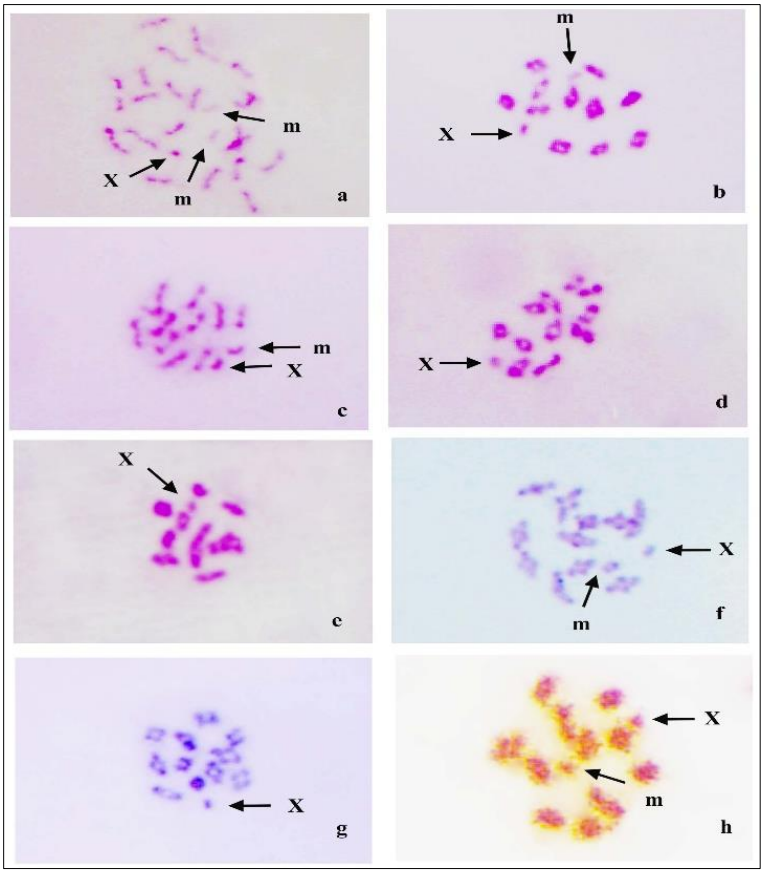


**Fig 1:** Normal complement, C bands and NOR's in *Anisopleura lestoides*: 1a-1e. Normal complement. 1a. spermatogonium metaphase 1b. diakinesis (n=13), 1c. metaphase-I (n=13), 1d. diakinesis (n=12), 1e. metaphase-I (n=12). 1f-1g. C-bands. 1f. diakinesis (n=13), 1g. diakinesis (n=12), 1h. NOR's. 1h. diakinesis.

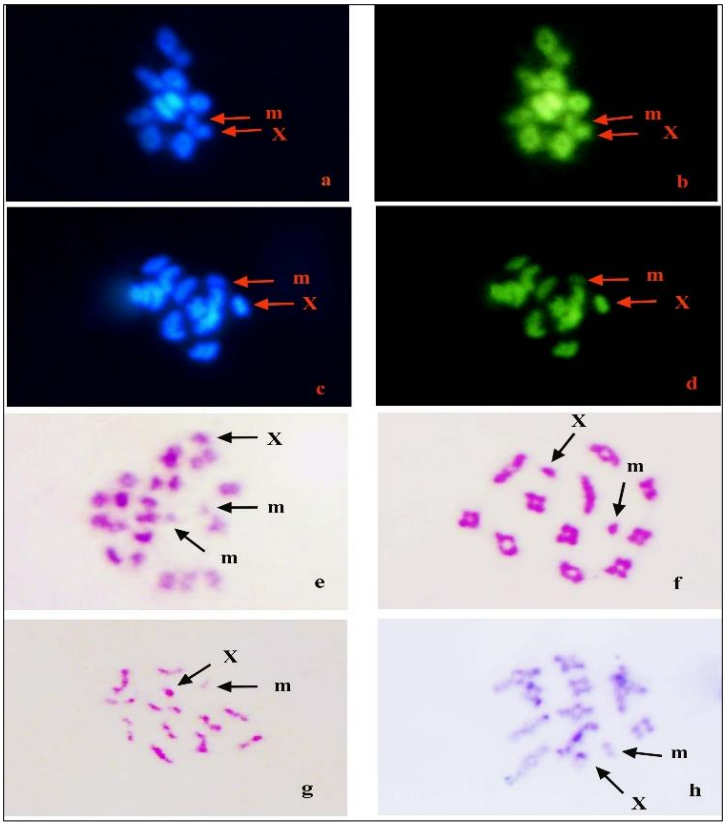


**Fig 2:** Sequence specific staining in Anisopleura lestoides and Normal complement, C bands in Bayadera indica: 2a-2d. sequence specific staining in Anisopleura lestoides. 2a, 2b. diakinesis 2c, 2d metaphase-I. 2e-2g. Normal complement in Bayadera indica. 2e. spermatogonial metaphase, 2f. diakinesis, 2g. metaphase-I. 2h. C-bands in Bayadera indica. diakinesis.

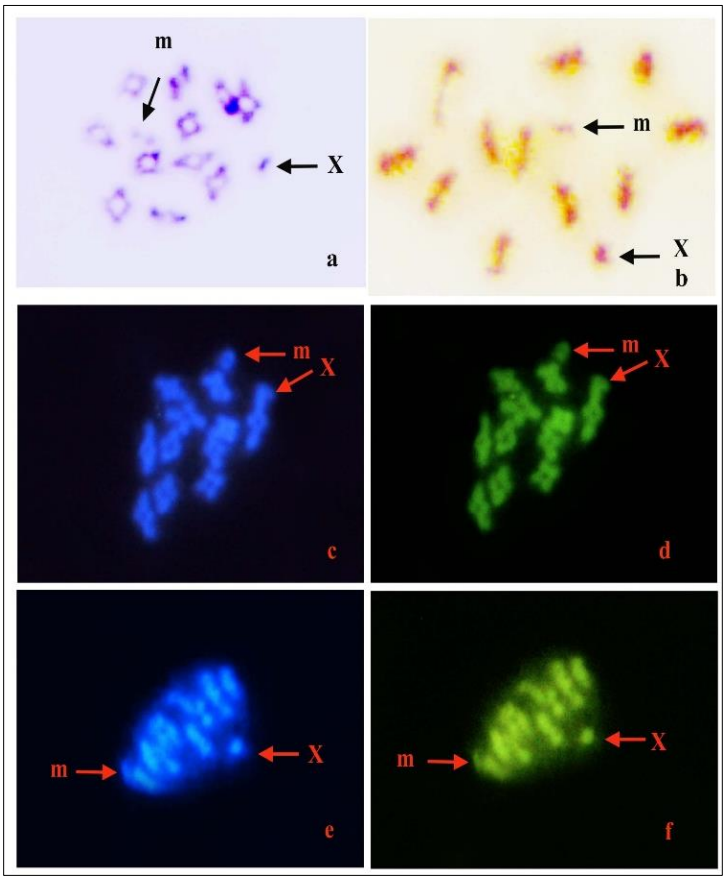


**Fig 3:** C bands and NOR's in *Bayadera indica*: 3a. C-bands. diakinesis. 3b. NOR's. metaphase-I. 3c-3f. sequence specific staining in *Bayadera indica*. 3c, 3d. diakinesis, 3e, 3f. metaphase-I.

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