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First cytogenetic report on four species of family libellulidae (Odonata: Anisoptera) from India

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Abstract
Chromosome complement of *Cratilla lineata*, *Hylaeothemis apicalis*, *Orthetrum chrysis* and *Zygonix irix* of family Libellulidae has been studied by conventional staining, C-banding, silver nitrate staining and sequence specific staining. Among these, *Cratilla lineata, Orthetrum chrysis* and *Zygonix irix* possess 2n (♂) = 25 as the chromosome number, while *Hylaeothemis apicalis* reveals 2n (♂) =23, which is originated by the fusion of X chromosome with an autosome pair as X chromosome is the largest element in the complement. Terminal C-bands and terminal NOR's are present on the autosomal bivalents, while X chromosome is entirely C-positive and NOR-positive in all the species, whereas m bivalent shows variation in distribution of C-heterochromatin and NOR's. Similarly, autosomal bivalents and X chromosome show more DAPI bright signal than CMA_3, while m bivalent possesses variation in AT-GC region specificity. Cytogenetically, all these species has been studied for the first time in the world.

Keywords: dragonfly, anisoptera, libellulidae, constitutive heterochromatin, nucleolar organizer regions, sequence specificity

Introduction

Family libellulidae is the largest family of suborder Anisoptera which includes 1035 species of 144 genera throughout the world and 91 species of 40 genera are present in India (Subramanian and Babu, 2017). Cytogenetic data pertains to 254 libellulid species, out of those, 41 species are from India (Kuznetsova and Golab, 2020). In family Libellulidae, chromosome number varies from 2n=6-47, while majority of the species possess the male chromosome complement 2n (♂) =25, which is the modal number of the family. Variation in chromosome number has been found in 47 libellulid species, which is due to autosomal fragmentations; fusion of autosomes/fusion of autosome with X chromosome resulted in neo-XY sex determination and presence/absence of m chromosomes in different geographical populations of species (Kuznetsova and Golab, 2020). So far, linear characterization of chromosomes based on C-banding on 35 species, Silver nitrate staining on 22 species and sequence specific staining on 19 species of family Libellulidae has been done (Thomas and Prasad, 1986; Frankovic and Jurecic, 1989; Prasad and Thomas, 1992; Diego *et al*., 2008; Walia *et al*., 2010; Lone, 2015; Kaur, 2016) [14, 3, 10, 2, 15, 9, 6]. Presently, Cytogenetic analysis based on structure and behavior of chromosome in the complement, distribution of C-bands, localization of NOR's and AT-GC rich regions have been done on *Cratilla lineata*, *Hylaeothemis apicalis*, *Orthetrum chrysis* and *Zygonix irix* for the first time.

Materials and methods

The adult male specimens of all the species were collected from the different regions of Kerala in the year 2018 (Table-I). Specimens were dissected in 0.67% saline solution in the field and testes were taken out. Testes were fixed in freshly prepared Carnoy's fixative for chromosomal preparations (3:1, absolute alcohol: glacial acetic acid). Later, slides were treated for conventional staining (Carr and Walker, 1961) [1], C-banding (Sumner, 1972) [13], silver nitrate staining (Howell and Black, 1980) and sequence specific staining (Rebagliati *et al*., 2003) [11]. Libellulid species were identified by consulting "The Fauna of British India, including Ceylon and Burma, Odonata Vol-III" (Fraser, 1936) [4].

Table 1: List of species with detailed information of localities

S.No.	Name of the species	Date of Collection	Location	State	Coordinates
1.	*Cratilla lineata* (Brauer, 1878)	05/05/2018	Mananthavady (Wayanad)	Kerala	11.8014° N 76.0044° E
2.	*Hylaeothemis apicalis* Fraser, 1924	O5/05/2018	Mananthavady (Wayanad)	Kerala	11.8014° N 76.0044° E
3.	*Orthetrum chrysis* (Selys, 1891)	04/04/2018	Quilandy (Kozikode)	Kerala	11.4429° N 75.6976° E
4.	*Zygonix irix* Selys,1869	06/05/2018	Pathanapuram (Kollam)	Kerala	9.0927° N 76.8612° E

Results

Conventional staining

During diakinesis and metaphase-I of *Cratilla lineata*, *Orthetrum chrysis* and *Zygonix irix*, 13 elements are visible, among these, 12 are autosomal bivalents and one is X chromosome, while in *Hylaeothemis apicalis*, 12 elements are seen which include 11 autosomal bivalents and one largest X univalent. m bivalent is present in all the species except in *Zygonix irix* (Figs, 1a,b; 2a,b; 3a,b; 4a,b).

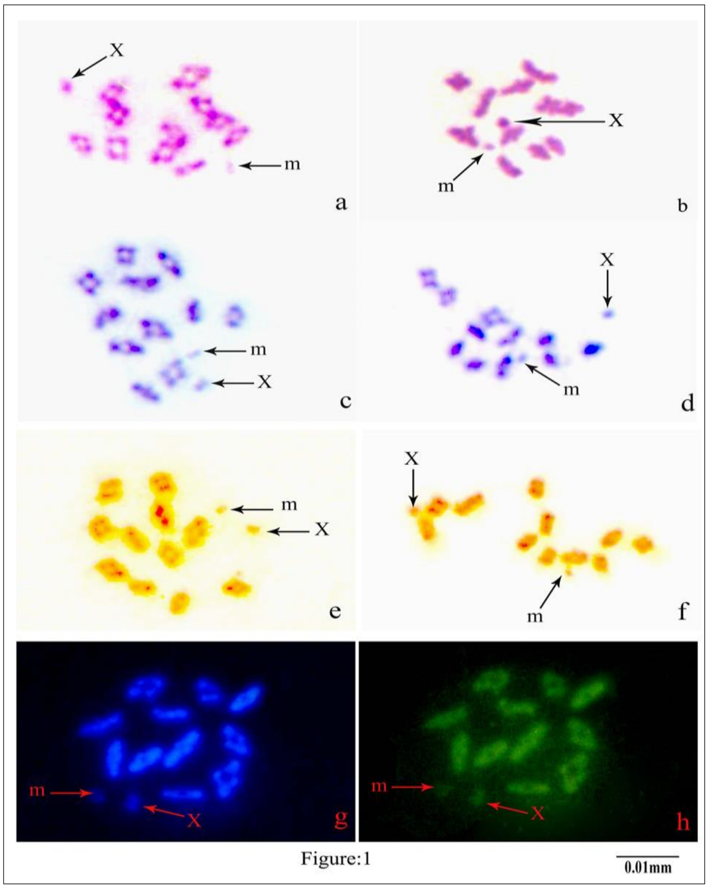

Fig 1: *Cratilla lineata*- Conventional staining (a:Diakinesis, b: Metapahse-I); C-banding (c:Diakinesis, d:Metaphase-I); Silver staining (e:Diakinesis, f:metaphase I); Sequence specific staining g-h: Diakinesis (g:DAPI staining; h:CMA3 staining). X and m marked with arrows. Bar= 0.01mm

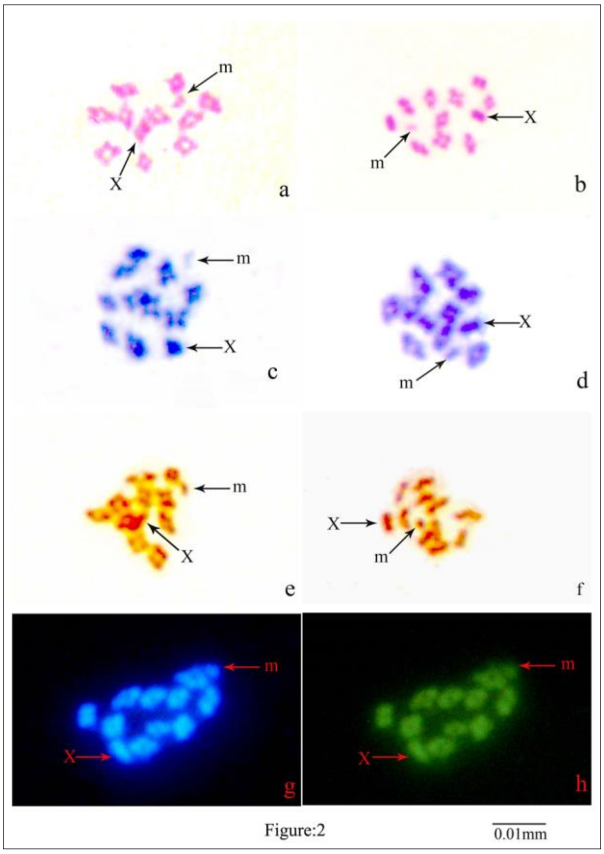

Fig 2: *Hylaeothemis apicalis* – conventional staining (a: Diakinesis, b: Metaphase-I), C-banding (c:Diakinesis, d: Metaphase-I), Silver nitrate staining (e: Pachytene, f:Diakinesis), Sequence specific staining g-h Diakinesis (g:DAPI staining; h:CMA3 staining). X and m marked with arrows. Bar= 0.01mm

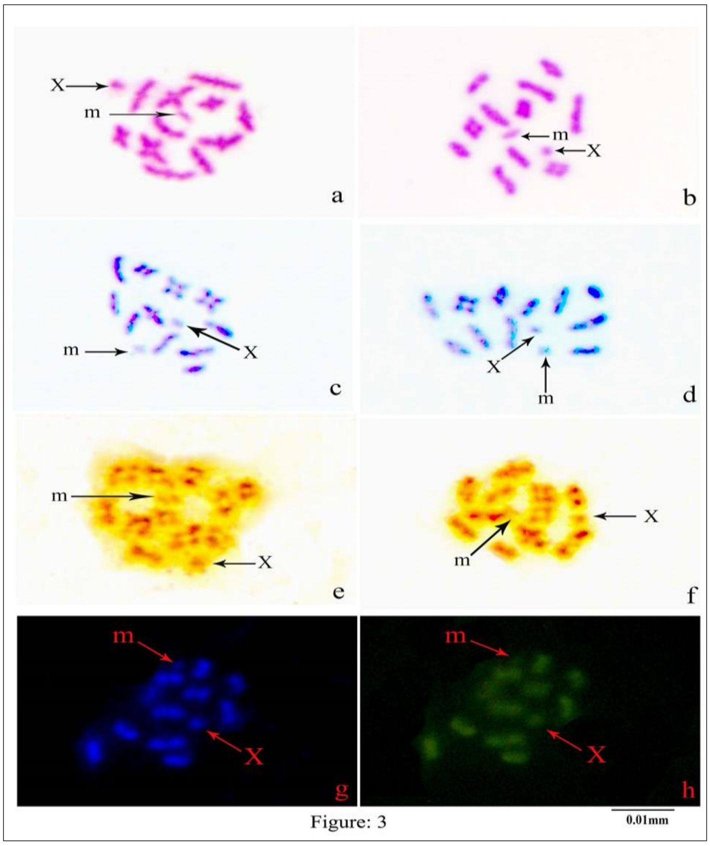

Fig 3: *Orthetrum chrysis* - Conventional staining (a:Diakinesis, b:Metaphase-I); C-banding (c:Late diakinesis, d:Metapahse-I); Silver staining (e:Diakinesis, f:metaphase-I); Sequence specific staining g-h: Spermatogonal metaphase (g:DAPI staining; h:CMA_3 staining). X and m marked with arrows. Bar= 0.01mm

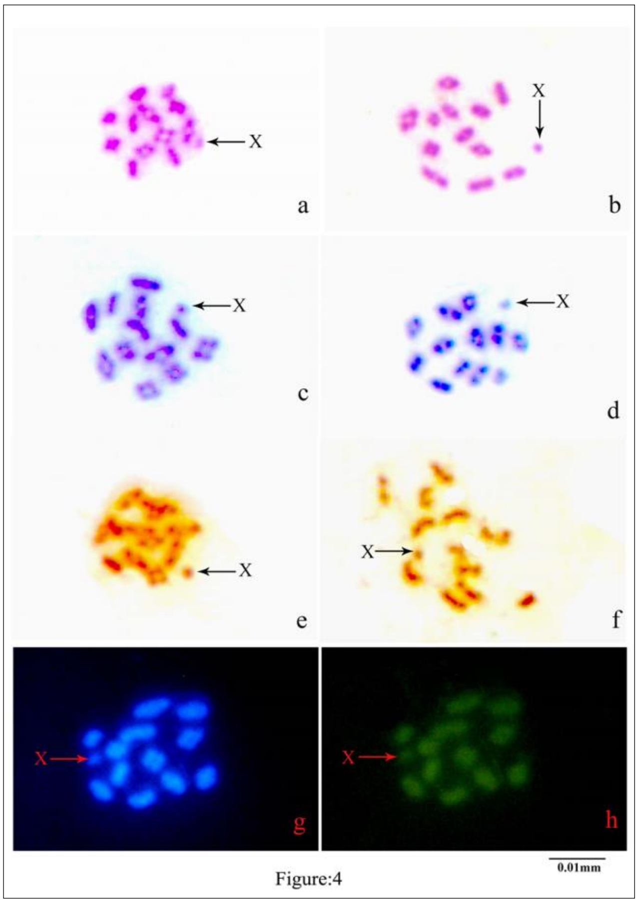

Fig 4: *Zygonis chrysis*: Conventional staining (a:Diakinesis b:Metapahse-I); C-banding (c:Diakinesis, d:metaphase-I); Silver staining (e:Diakinesis, f:metaphase-I); Sequence specific staining g-h:Diakinesis (g:DAPI staining; h:CMA_3 staining) X and m marked with arrows. Bar= 0.01mm

C-banding

During the diakinesis and metaphase-I, autosomal bivalents show large terminal C-bands and X chromosome is entirely C-positive in all the species. m bivalent is C-negative in *Cratilla lineata* and *Orthetrum chrysis*, but C-positive in *Hylaeothemis apicalis* (Figs, 1c,d; 2c,d; 3c,d; 4c,d).

Silver nitrate staining

In the diakinesis and metaphase-I, autosomal bivalents possess terminal NOR's and X chromosome is NOR-positive in all the species. m bivalent is NOR-positive in *Hylaeothemis apicalis*, while NOR-negative in *Cratilla lineata* and *Orthetrum chrysis* (Figs, 1d,e; 2d,e; 3d,e; 4d,e).

Sequence specific staining

During the diakinesis of *Cratilla lineata*, *Hylaeothemis apicalis* and *Zygonix irix* and metaphase –I of *Orthetrum chrysis*, autosomal bivalents show more DAPI bright signal than CMA_3 at the chiasmatic ends, while m bivalent possesses dull signal for both DAPI and CMA_3 in *Cratilla lineata* and *Orthetrum chrysis* and DAPI bright in *Hylaeothemis apicalis*. X chromosome also reveals DAPI bright signals in all the species (Figs, 1g,h; 2g,h; 3g,h; 4g,h).

Discussion

The modal number of the family Libellulidae is 2n=25m, as it is present in a majority of the species. Moreover, during the course of karyotypic evolution in the family, chromosome number varies from n=3-18 and sex determination from X0 to neo-XY. Cytogenetic data pertains to 254 libellulid species, out of these, 41 species are from India. Variation in chromosome number has been found in 47 libellulid species, which is due to autosomal fragmentations and fusion of autosomes/fusion of autosome with X chromosome resulted in neo-XY sex determination and presence/absence of m chromosomes in different geographical populations of species (Kuznetsova and Golab, 2020).

In the present study, three species *Cratilla lineata*, *Orthetrum chrysis* and *Zygonix irix* reveals the chromosome complement 2n=25, while *Hyaleothemis indica* possesses the 2n=23 and X0 (♂)/XX (♀) type sex determination mechanism. X chromosome is the smallest element in the whole complement of all the species except in *Hylaeothemis apicalis* in which it is largest element which resulted by the fusion of sex chromosome with an autosome pair. Earlier, in 35 libellulid species, reduced chromosome number either due to the fusion between autosomes or fusion between autosome and X chromosome resulted in neo-XY sex determination has been reported (Kuznetsova and Golab, 2020). In Odonata, fusions favour the survival value and reproductive capabilities of species (Kiauta, 1969). m-chromosomes are present in all the species except in *Zygonix irix.*

So far, C-banding has been done on 35 libellulid species and heterochromatic block are mostly present on both telomeric regions of autosomes, while sex chromosome and m chromosome show variations (Thomas and Prasad, 1986; Frankovic and Jurecic, 1989; Prasad and Thomas, 1992; Walia *et al.*, 2010; Lone, 2015 and Kaur, 2016) [14, 3, 10, 15, 9, 6]

Similarly, during the present study, all autosomal bivalents show large terminal C-bands and X chromosome is entirely C-positive in all the species, while m bivalent is C-negative in *Cratilla lineata* and in *Orthetrum chrysis*, but it is C-positive in *Hylaeothemis apicalis*.

Silver nitrate staining has been done on 22 libellulid species. Majority of NOR's are found to be associated with terminal ends of autosomal bivalents and X chromosome shows variation in distribution of NOR's (Walia *et al.*, 2010; Lone, 2015; Kaur, 2016) [14, 9, 6]. Presently, autosomal bivalents show terminal NOR's and X chromosome is NOR- positive in all the species. M bivalent is NOR-positive in *Hylaeothemis apicalis* and it is NOR-negative in *Cratilla lineata* and *Orthetrum chrysis*.

Sequence specific staining has been performed on 19 libellulid species to characterize the C-heterochromatin in terms of A-T and G-C richness. Majority of the species show superimposition of AT-GC rich regions (Diego *et al*., 2008; Kaur, 2016) [2, 6]. Presently, autosomal bivalents show more DAPI bright signal than CMA_3 at the chiasmatic ends which correspond to the C-bands and NOR's. m bivalent shows dull signal for both DAPI and CMA_3 in *Cratilla lineata* and *Orthetrum chrysis* as it is C-negative and NOR-negative, while DAPI bright in *Hylaeothemis apicalis* as it is C-positive and NOR-positive. X-chromosome is more DAPI bright than CMA_3 as it is C-positive and NOR-positive.

Conclusion

Cytogenetic characterization of chromosomes of *Cratilla lineata* (2n=25m), *Hylaeothemis apicalis* (2n=23m), *Orthetrum chrysis* (2n=25m) and *Zygonix irix* (2n=25) has been done for the first time. All the species possess XX/X0 sex determining machanism. The decrease in chromosome number of *Hylaeothemis apicalis* resulted by the fusion of X chromosome with an autosome pair as it is the largest chromosome in the complement. All the autosomal bivalents show large terminal C-bands and terminal NOR's and X chromosome is entirely C-positive and NOR-positive in all the species, while m bivalent possesses variation in distribution of C-heterochromatin and NOR's. In all the species, autosomal bivalents and X univalent show more DAPI bright signal than CMA_3, while m bivalent possesses dull signal for both DAPI and CMA_3 in *Cratilla lineata* and in *Orthetrum chrysis*, while DAPI bright in *Hylaeothemis apicalis*. Results of sequence specific staining are corresponding to the results of C- banding and silver nitrate staining.

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