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Cytological Review and First Cytogenetic Report on Three Species of Family Macromiidae (Odonata: Anisoptera) from India

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Abstract: Cytological data of family Macromiidae based on chromosome number and sex determination has been reviewed and cytogenetic investigations on *Epophthalmia vittata* Burmeister, 1839; *Macromia ellisoni* Fraser, 1924 and *Macromia flavicincta* Selys, 1874 have been done using conventional staining, C- banding, silver nitrate staining and sequence specific staining. Macromiid species were captured from Maharashtra (Nagpur) and Kerala (Kuttiadi river and Vatakara) states of India. All the species possess 2n (♂) = 25m, which is the type number of family with X0-XX type sex determination. All the autosomal bivalents including large bivalent present in *Epophthalmia vittata* and *Macromia ellisoni* show terminal C-bands and NOR's, while X chromosome is C- positive and NOR rich, whereas m bivalent is C-negative and NOR- negative. In the sequence specific staining, all autosomal bivalents including X chromosome possess overlapping DAPI/CMA_3 signals. Cytogenetically, all the three species have been studied for the first time.

Keywords: Anisoptera, Macromiidae, Constitutive heterochromatin, NOR's, Sequence specific staining

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Introduction

Dragonflies of family Macromiidae are known as river cruisers or macromiids. These are morphologically same as the dragonflies of family Aeshnidae in size, but their green eyes are rarely met at top (Fraser, 1936). Globally, family Macromiidae contains 4 genera and 123 species, while only 2 genera, *Epophthalmia* Burmeister, 1839 and *Macromia* Rambur, 1842 and 17 species are present in India (Subramanian and Babu, 2017). So far, cytogenetic data is available only on 6 species of family Macromiidae including two species from India, which is less than 5% of taxonomically known species (Dasgupta, 1957; Cruden, 1968; Kiauta, 1977; Katatani, 1987; Perepelov and Bugrov, 2001; Walia and Chahal, 2018). Majority of the species possess 2n= 25 with X0/XX sex determination mechanism. In the present study, chromosomal analyses of *Epophthalmia vittata*, *Macromia ellisoni* and *Macromia flavicincta* based on conventional staining, C-banding, silver nitrate staining and sequence specific staining have been done. All the species possess 2n= (♂) 25m with X0(♂)/XX (♀) sex determination. Structure and behaviour of

Table 1: Cytological data of family Macromiidae

S. No.	Species	Chromosome Complement	Sex Determination	Locality	References
1	*Didymops transversa* (Sey, 1839)	n=13 (m)	XO/XX	U.S.A.	Cruden, 1968
2	*Epophthalmia frontalis frontalis* Selys, 1871	n=13 (m)	XO/XX	India	Dasgupta, 1957
3	*Epophthalmia vittata* Burmeister, 1839	n=13 (m)	XO/XX	India	Present study
4	*Macromia amphigenia* Selys, 1871	n=13	XO/XX	Russia	Perepelov and Bugrov, 2001
5	*Macromia daimoji* Okumura, 1949	n=13	XO/XX	Japan	Katatani, 1987
6	*Macromia ellisoni* Fraser, 1924	n=13 (m)	XO/XX	India	Present study
7	*Macromia flavicincta* Selys, 1874	n=13 (m)	XO/XX	India	Present study
8	*Macromia magnifica* (McLachlan, 1874)	n= 13 (m) n= 13	XO/XX	U.S.A.	Cruden, 1968
9	*Macromia moorei* Selys, 1874	n=13 (m)	XO/XX	Nepal India	Kiauta, 1977 Walia and Chahal (2018)

chromosomes during meiosis, presence of constitutive heterochromatin, localization of Nucleolar Organizer Regions and distribution of AT and GC rich regions in these species have been studied and compared. The list of the cytologically studied species of the family Macromiidae has been updated to 9 species (Table 1).

Materials and Methods

Adult male specimens were collected from the states of Maharashtra and Kerala of India during the pre- and post-monsoon seasons of the year 2019 (Table 2). Specimens were dissected in 0.67 % saline solution in the field, testes were removed and fixed in freshly prepared Carnoy's fixative then teased on grease/dust free slides. Slides were processed for conventional staining (Carr and Walker, 1961), C-banding (Sumner, 1972), silver nitrate staining (Howell and Black, 1980) and sequence specific staining (Rebagliati *et al*., 2003). Relevant meiotic stages were photomicrographed for further cytogenetical investigations.

Results

Conventional staining: Diakinesis of all the species possess 13 elements, among these, 12 are autosomal bivalents including m bivalent and small X chromosome. All the bivalents show cross shaped structure due to the presence of single chiasma per bivalent. Moreover, *Epophthalmia vittata* and *Macromia ellisoni* possess one large bivalent in the complement, but it is absent in *Macromia flavicincta* (Figs. 1a, 2a, 3a). During metaphase-I, autosomal bivalents appear rod shaped due to condensation and terminalization of chiasmata, while X chromosome and m bivalent are clearly distinct and large bivalent in *Epophthalmia vittata* and *Macromia ellisoni* is also distinguishable (Figs. 1b, 2b, 3b).

C- banding: In C-banded diakinesis, all the autosomal bivalents including large bivalent show dark terminal C-bands, while m bivalent is C-negative and X chromosome is C-positive in all the species (Figs. 1c, 2c, 3c).

Table 2: Collection data on three species of family Macromiidae

S. No.	Species	Collected site	Longitude	Latitude	Altitude
1	*Epophthalmia vittata* Burmeister, 1839	Zilpi lake, Nagpur (Maharashtra)	21.1458° N	79.0882° E	310m
2	*Macromia ellisoni* Fraser, 1924	Water body, Koyilandy (Kerala)	11.4429° N	75.6976° E	122m
3	*Macromia flavicincta* Selys, 1874	Water body, Vatakara (Kerala)	11.6016° N	75.5920° E	124m

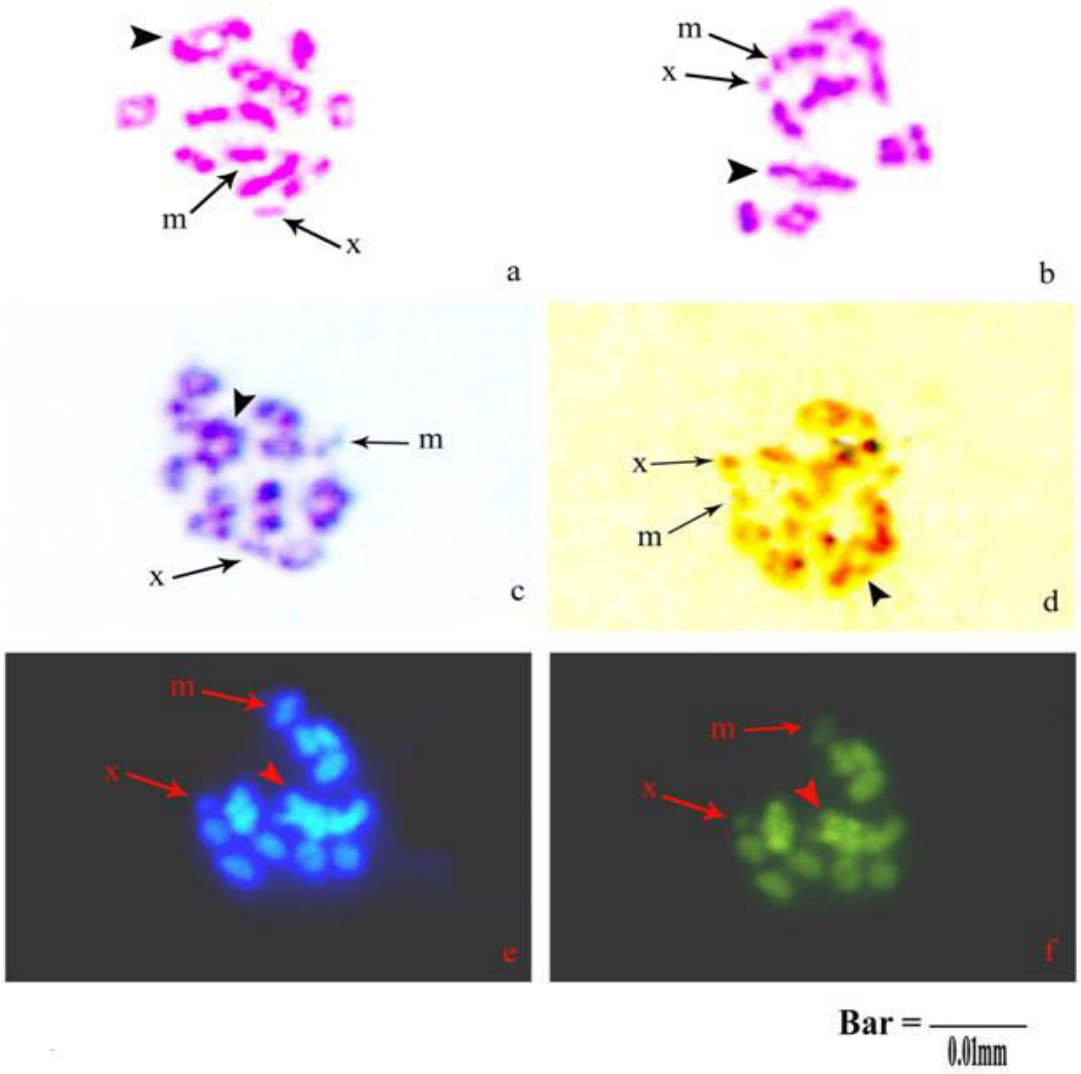

Fig. 1: Normal complement, C-bands, NOR's, Sequence specific regions in *Epophthalmia vittata* Burmeister, 1839: Normal complement (1a) Diakinesis, (1b) Metaphase-I, C-banding (1c) Diakinesis, AgNOR staining (1d) Diakinesis, Sequence specific staining (1e) Diakinesis DAPI (1f) Diakinesis CMA_3. Arrows show the X chromosome and m bivalent. Arrowhead shows the large bivalent. Bar = 0.01 mm.

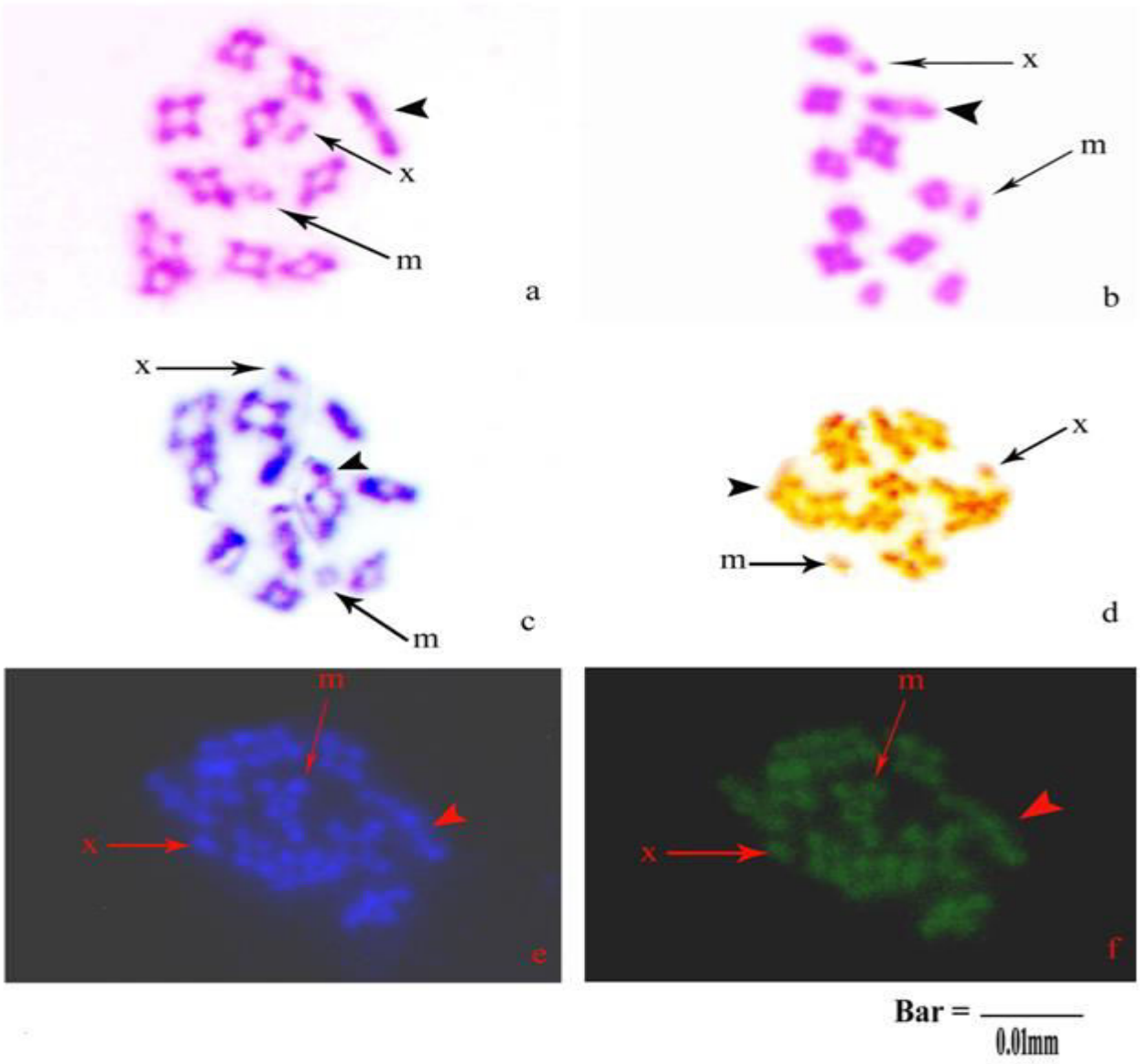

Fig. 2: Normal complement, C-bands, NOR's, Sequence specific regions in *Macromia ellisoni* Fraser, 1924: Normal complement (2a) Diakinesis, (2b) Metaphase-I, C-banding (2c) Diakinesis, AgNOR staining (2d) Diakinesis, Sequence specific staining (2e) Diakinesis DAPI, (2f) Diakinesis CMA_3. Arrows show the X chromosome and m bivalent. Arrowhead shows the large bivalent. Bar = 0.01 mm.

Silver nitrate staining: In AgNOR treated diakinesis of all the species, autosomal bivalents including large bivalent show light/dark terminal NOR's on one side/both the sides, whereas m bivalent is NOR - negative and X chromosome is NOR rich (Figs.1d, 2d, 3d).

Sequence specific staining: In all the species, during diakinesis, autosomal bivalents including m bivalent show overlapping DAPI and CMA_3 signals at the terminal ends, while large bivalent in *Epophthalmia vittata* and *Macromia ellisoni* possesses more DAPI bright signals and X chromosome shows both DAPI and CMA_3 bright signals (Figs.1e, 1f, 2e, 2f, 3e, 3f).

Discussion

Taxonomically, family Macromiidae contains 123 species under four genera *Didymops*, *Epophthalmia*, *Macromia* and *Phyllomacromia*,

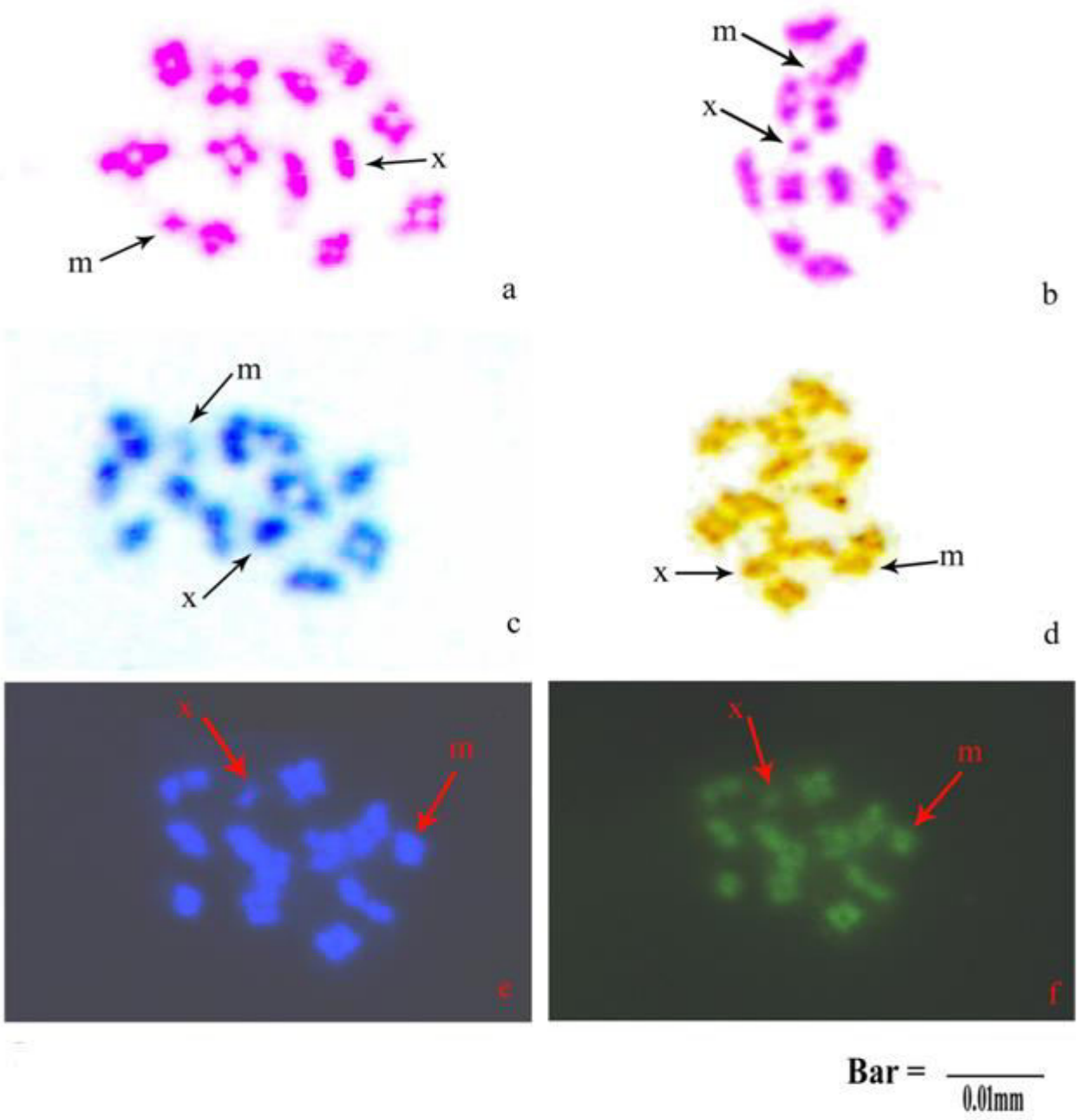

Fig. 3: Normal complement, C-bands, NOR's, Sequence specific regions in *Macromia flavicincta* Selys, 1874: Normal complement (3a) Diakinesis, (3b) Metaphase-I, C-banding (3c) Diakinesis, AgNOR staining (3d) Diakinesis, Sequence specific staining (3e) Diakinesis DAPI (3f) Diakinesis CMA_3. Arrows show the X chromosome and m bivalent. Arrowhead shows the large bivalent. Bar = 0.01 mm.

while only two genera *Epophthalmia* and *Macromia* representing 17 species are present in India (Subramanian and Babu, 2017). Cytogenetically, 6 species of family Macromiidae have been studied and majority of species possess 2n = 25, which is considered as type number of the family with X0/XX sex determination. Genus *Epophthalmia* includes 6 species worldwide, while 4 species are present in India (Subramanian and Babu, 2017). So far, only one species *Epophthalmia frontalis frontalis* with n = 13m with X0/XX sex determination has been described (Dasgupta,

1957). Presently, one more species *Epophthalmia vittata* with same chromosome number, n= 13m with X0/XX sex determination has been studied. Moreover, this species is characterized by the presence of large bivalent in the complement, which is considered as the species specific character.

Genus *Macromia* includes 80 species worldwide, while 14 species are present in India (Subramanian and Babu, 2017). Cytogenetically, 4 species have been described worldwide. All the species possess n= 13, but m chromosomes are present in *Macromia moorei* (Walia and Chahal, 2018), while absent in *Macromia amphigenia* (Perepelov and Bugrov, 2001) and *Macromia daimoji* (Katatani, 1987). On the other hand, *Macromia amphigenia* possess both the complements n= 13m and n= 13. Presently, two more species *Macromia ellisoni* and *Macromia flavicincta* have been studied and both the species possess n= 13m with X0/XX sex determination. Moreover, *Macromia ellisoni* is characterized by the presence of one large bivalent which is considered as the species specific character.

Linear characterization of chromosomes of *Epophthalmia vittata*, *Macromia ellisoni* and *Macromia flavicincta* has been done. Till date, chromosomes of only one species *Macromia moorei* has been linearly characterized (Walia and Chahal, 2018). In all the species, C-bands and NOR's are mostly present at the terminal ends of the autosomal bivalents, while m bivalent is C-negative and NOR-negative, whereas X chromosome is C-positive and NOR rich. The complement of all the species possess overlapping DAPI/ CMA_3 signals, which confirms the results of C-banding and NOR staining as C - bands correspond to AT rich regions and NOR's correspond to GC rich regions. Cytogenetic analysis on *Epophthalmia vittata*, *Macromia ellisoni* and *Macromia flavicincta* have been done for the first time and list of cytologically studied species of family Macromiidae has been updated to 9 species.

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