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Cytogenetic Characterization Of *Macromia Moorei* Selys, 1874 Of Family Macromiidae (Odonata:Anisoptera) From India By C-Banding, Silver Nitrate Staining And Sequence Specific Staining

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Abstract: **Live adult male specimens of *Macromia moorei* Selys, 1874 of family Macromiidae have been collected from Andretta, Himachal Pradesh (India). Male germ cell chromosomes of the species have been described on the basis of conventional staining, C-banding, silver nitrate staining and sequence specific staining. The species possesses 2n (♂) =25m, as the chromosome number and XO (♂)/XX (♀) type sex determination. Dark terminal C-bands are present on all the autosomal bivalents, while m bivalent shows small terminal C-bands and X chromosome is C-positive throughout the length. Terminal light/dark NOR's are present on all autosomal bivalents including m bivalent, while X chromosome also possesses terminal NOR. During sequence specific staining, all the autosomal bivalents show prominant terminal DAPI and CMA3 bright regions, while m bivalent possesses less bright DAPI and CMA3 signals and X chromosome possesses both DAPI and CMA3 regions. Linear characterization of chromosomes of *Macromia moorei* has been done for the first time.**

Keywords: **Macromiidae, Chromosomes, Odonata, Anisoptera, Conventional Staining, C-banding, Silver nitrate staining, Sequence specific staining.**

I. INTRODUCTION

Order Odonata is one of the ancient groups of insects. Fossil evidences suggest that origin of this group dates back to Permian (250 million years BP). Globally, 6,256 species in 686 genera of odonates are known, while 487 species, 27 subspecies referable to 152 genera and 18 families are present in India. Dragonflies of family Macromiidae tend to fly over water bodies and roads straight down the middle. They are similar to dragonflies of family Aeshnidae in size, but their eyes are green and rarely meet at top of the head. Family Macromiidae contains 4 genera and 123 species worldwide, while only 2 genera, *Epophthalmia* Burmeister, 1839 and *Macromia* Rambur, 1842 having 17 species are present in India (Subramanian and Babu, 2017). Earlier, four species of family Macromiidae *Epophthalmia frontalis frontalis*, *Didymops transversa*, *Macromia magnifica* and *Macromia moorei* have been studied cytogenetically (Dasgupta, 1957; Cruden, 1968; Kiauta, 1977). All the species possess 2n= 25m, while one population of *Macromia magnifica* possesses 2n=25, without m chromosome (Cruden, 1968).

In the present study, *Macromia moorei* possesses 2n (♂) =25m, with XO (♂)/XX (♀) type sex determination. All the autosomal bivalents except m bivalent possess dark terminal C-bands and light NOR's. Moreover, m bivalent shows light terminal C-bands and NOR's, while X chromosome is C-positive and possesses terminal NOR. Sequence specific staining confirms the results of C-banding and silver nitrate staining as all the bivalents and X chromosome possess both DAPI and CMA3 bright signals.

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II. MATERIALS AND METHODS

Live adult male specimens were collected from Andretta, Himachal Pradesh in the month of September, 2015. Specimens were dissected in 0.67% saline solution in the field and testes were taken out. Subsequently, the testes were put in sodium citrate (0.9%) for 45 minutes then fixed in freshly prepared Carnoy's fixative (3: 1, absolute alcohol: acetic acid glacial) for 15 minutes. Two more changes in the fixative, each of 15 minutes duration were given. After this, testes were teased on the grease free slides and slides were air dried.

For the conventional staining, the prepared slides were stained in Carbol fuchsin for 3-4 hours as suggested by Carr and Walker (1961). The technique suggested by Sumner (1972) was followed for the detection of constitutive heterochromatin. To study the localization of Nucleolar Organiser Regions (NOR's) the technique suggested by Howell and Black (1980) was employed. For the sequence specificity, the technique suggested by Rebagaliati *et al.* (2003) was followed. Relevant meiotic and mitotic stages were microphotographed.

III. RESULTS

Conventional Staining:

Spermatogonial metaphase possesses 25 elements, out of these, 24 are autosomes and one 2nd smallest element is the X chromosome. Autosomes also include small pair of m chromosomes (Fig. 1a). In the diplotene and diakinesis, 13 elements are visible, out of these, 12 are autosomal bivalents and one is X chromosome. All the autosomal bivalents including m bivalent are cross shaped due to the presence of single chiasma per bivalent (Figs. 1b, 1c). During metaphase I, the bivalents show terminalisation of chiasma and condensation, while X chromosome is oval shape (Fig. 1d). In the metaphase-II, chromosomes are half the size of metaphase I elements (Fig. 1e).

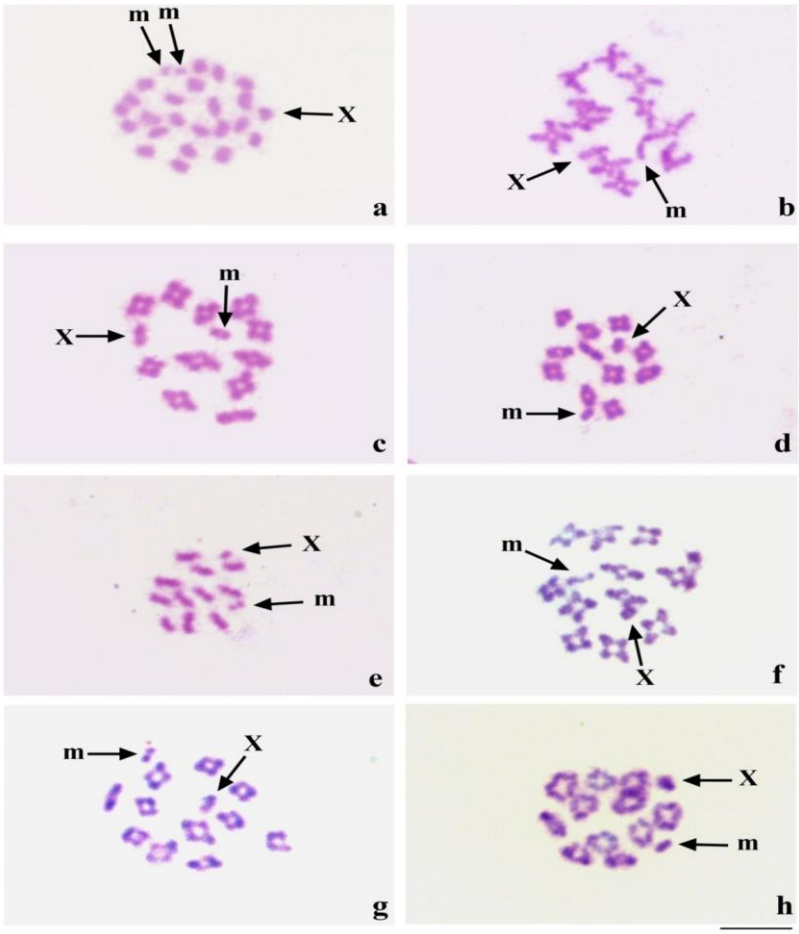

Fig 1: a-e, Conventional staining, f-h, C-banding, a spermatogonial metaphase, b diplotene, c diakinesis, d metaphase I, e metaphase II, f-h diakinesis. X and m marked with arrows. Bar= 0.01mm

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Banding patterns of chromosomes:

In the C-banding, all the autosomal bivalents show dark terminal C-bands, while X chromosome is positively heterochromatic and m bivalent shows light terminal C-bands during diakinesis (Figs. 1f,g,h). In the silver nitrate staining, darkly stained nucleolus is visible in all the interphase cells (Fig. 2a). All the autosomal bivalents including m bivalent possess light terminal NOR's, while X chromosome possess terminal NOR on one side during metaphase-I (Fig. 2b). In the sequence specific staining, terminal DAPI and CMA3 bright regions are visible on all the chromosomes, while X possesses DAPI and CMA3 bright signals during spermatogonial metaphase (Figs. 2c, 2d). All the autosomal bivalents show prominant terminal DAPI and CMA3 bright regions, while m bivalent possesses less bright signals for both the dyes and X chromosome possesses both DAPI and CMA3 bright regions (Figs. 2e,f,g,h).

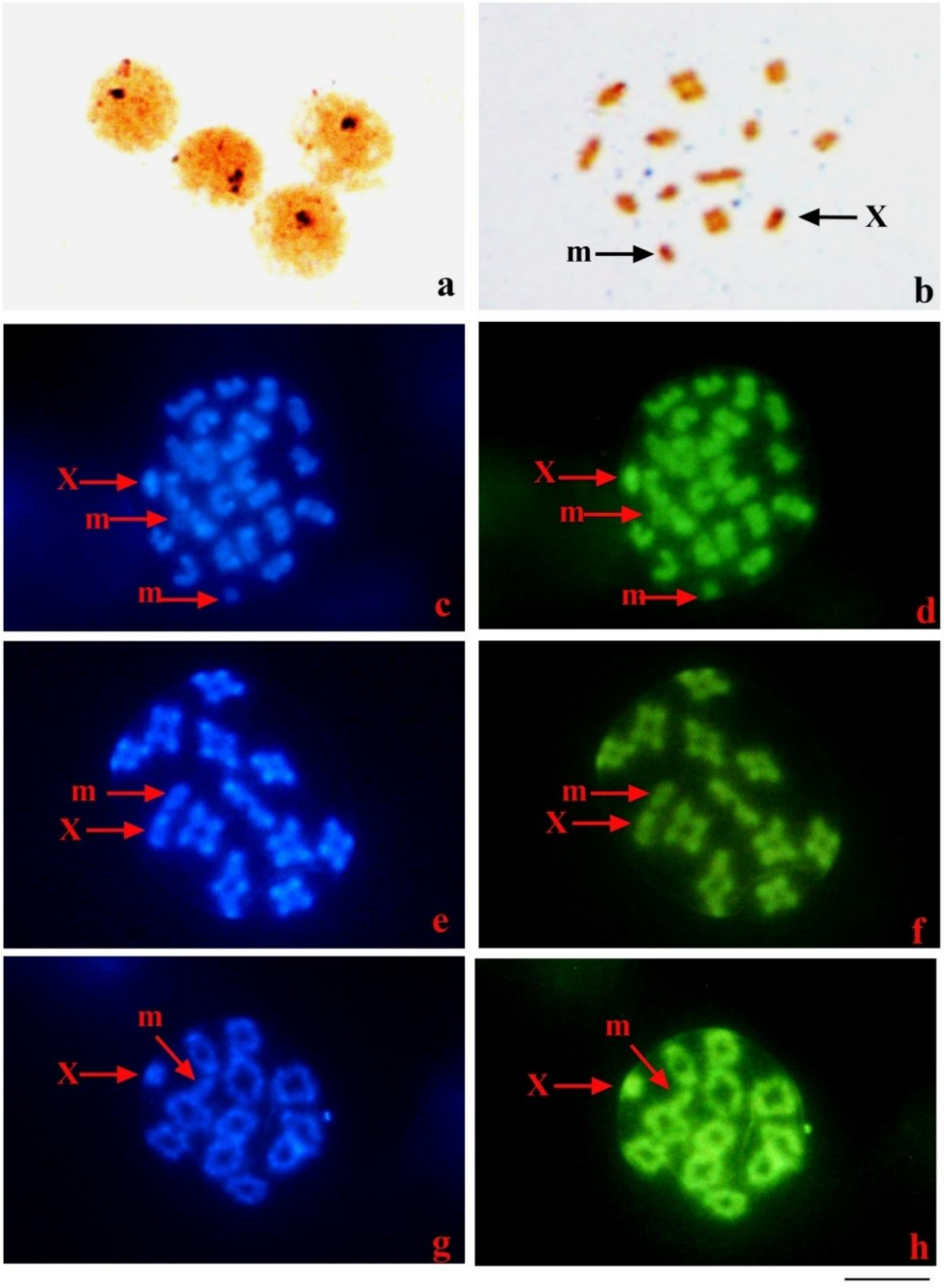

Fig 2: a-b, silver nitrate staining, c-h, sequence specific staining c,e,g, DAPI staining, d,f,h, CMA3 staining, a, interphase cell, b, metaphase I, c,d spermatogonial metaphase, e,f,g,h diakinesis. X and m marked with arrows. Bar= 0.01mm

IV. DISCUSSION

Taxonomically, family Macromiidae includes 4 genera and 125 species worldwide, while only 2 genera, *Epophthalmia* Burmeister, 1839 and *Macromia* Rambur, 1842 with 17 species are present in India (Subramanian and Babu, 2017). Type number of the family is 2n=25 as it is present in all the four studied species (Table I).

Table 1: Cytogenetic data of family Macromiidae

S. No.	Species	Chromosome complement	Sex detemination	Locality	References
1.	*Didymops transversa* (Say, 1839)	2n= 25 (m)	X0/XX	U.S.A.	Cruden, 1968
2.	*Epophthalmia frontalis frontalis* Selys, 1871	2n= 25 (m)	X0/XX	India	Dasgupta, 1957
3.	*Macromia magnifica* (McLachlan, 1874)	2n= 25 (m); 2n=25	X0/XX	U.S.A.	Cruden, 1968
4.	*Macromia moorei* Selys, 1874	2n= 25 (m)	X0/XX	Nepal Present study	Kiauta, 1977

Moreover, Cruden (1968) compared the two populations of *Macromia magnifica* on the basis of m chromosomes. He reported 2n=25m, in one population, while 2n=25 without m chromosomes in other population. Earlier, *Macromia moorei* has been studied by Kiauta (1977) from Nepal with chromosome number 2n=25m, X0/XX sex determination. Present results on the same species are in accordance to the earlier study but the species was collected from entirely a different locality (Andretta, Himachal Pradesh).

Few reports are available on linear characterization of chromosomes in sub order Anisoptera (Thomas and Prasad, 1986; Francovic and Jurecic, 1989; Prasad and Thomas, 1992; Perepelov *et al*., 1998, 2001; Perepelov and Bugrov, 2001, 2002; Nokkala *et al*., 2002; Grozeva and Marinov, 2007; De Gennaro *et al*., 2008, Walia *et al*., 2011, Walia and Chahal, 2014; Walia *et al*., 2016 and Kuznetsova *et al*., 2018). In the family Macromiidae, linear characterization of chromosomes in any species has not been reported. In *Macromia moorei*, all the autosomal bivalents possess dark terminal C-bands except m bivalent which shows small terminal C-bands and X chromosome is C-positive. The results of C-banding are in accordance to the results of sequence specific dye DAPI, which indicate the presence of AT rich regions. Similarly, in the silver nitrate staining, terminal light/dark NOR's present on all autosomal bivalents including m bivalent and terminal NOR on X chromosome correspond to the results of CMA3 dye, which stains GC rich regions. Presence of both DAPI and CMA3 signals at terminal positions indicates that the AT and GC rich regions are interspersed in *Macromia moorei* species.

V. CONCLUSION

Linear characterization of chromosomes of *Macromia moorei* has been done for the first time. The chromosome number is 2n=25m with X0/XX sex mechanism. All the bivalents and X chromosome possess both DAPI and CMA3 bright signals in sequence specific staining, which depicts the heterochromatin regions are showing interspersed AT and GC rich regions.

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