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Cytogenetic report on *Gynacanthaeschna sikkima* from India (Odonata: Aeshnidae)

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Abstract. Spermatogonial and primary spermatocyte chromosomes of *Gynacanthaeschna sikkima* (Karsch, 1891) collected from Dalhousie (Himachal Pradesh, India) are described cytogenetically for the first time. The species possesses 2n (♂) = 25 as the chromosome number and X0(♂)/XX(♀) type sex determining mechanism. The chromosome number is less than the modal number (2n = 27) of the family which originates from by the fusion of autosomes. All the autosomal bivalents except m bivalent show terminal C-bands while large autosomal bivalent possesses two interstitial and terminal C-bands. X chromosome shows large C-band only on one side. Similarly, terminal NOR bands are present on the one side of 9 autosomal bivalents including m bivalent while X chromosome possesses large interstitial NOR band.

Further key words. Dragonfly, Anisoptera, chromosomes, C-banding, silver nitrate staining.

Introduction

Taxonomically, 456 species of Aeshnidae referable to 51 genera are known worldwide, while 46 species referable to 13 genera are present in India (Subramanian 2014). In the Aeshnidae most of the cytogenetic work pertains to the genera *Aeshna* and *Anax* (Lefevre & McGill 1908; Mola & Papeschi 1994; Mola 1995; Walia & Sandhu 1999), while a few reports on the genera *Hemianax, Planaeschna, Cephalaeschna, Gynacantha* and *Corphaeschna* are also available (Makalovskaja 1940; Oksala 1943, 1944; Omura 1957; Seshachar & Bagga 1962; Cumming 1964; Cruden 1968; Kiauta 1968, 1970, 1972, 1975, 1979; Hung 1971; Tyagi

1982; Tyagi & Sangal 1982; Thomas & Prasad 1986; Walia 2007). The typical chromosome number of the family is 2n = 27. However, variations from this number (14–27) have also been reported in few species (Cumming 1964; Seshachar & Bagga 1962; Hung 1971; Kiauta 1967, 1971, 1975; Ferreira et al. 1979; Tyagi 1978a, b, 1982, 1986; Kiauta & Kiauta 1982; Mola & Papeschi 1994; Sandhu & Malhotra 1994; Mola 1995; Walia & Sandhu 1999; Walia 2007). The reduction in number is due to the fusion of autosome with autosome and autosome with sex chromosome. In the present study, chromosomal analyses by conventional staining, C-banding and silver nitrate staining have been done on *Gynacanthaeschna sikkima* (Karsch, 1891). This is the only representative of the genus present in the Indian subcontinent. The chromosome complement of the species is 2n(♂) = 25m with X0(♂)/XX(♀) type sex determination. The structure and behaviour of chromosomes during meiosis, detection of constitutive heterochromatin and localization of NOR have been investigated.

Materials and methods

Two adult male specimens of *Gynacanthaeschna sikkima* were collected alive near the water fall at Panchpula, Dalhousie, Himachal Pradesh, India, in September 2013. Specimens were dissected in 0.67 % saline solution (sodium chloride in distilled water) in the field and testes were taken out. Subsequently, the testes were put in sodium citrate (0.9 %) for 45 minutes and then fixed in freshly prepared Carnoy's fixative (3 parts absolute alcohol : 1 part glacial acetic acid) for 15 minutes. Two more changes in the fixative, each of 15 minutes duration were given. After this, the testes were teased apart on ten grease-free slides and the slides were air dried.

For the conventional staining, the prepared slides were stained in Carbol fuchsin for 3–4 hours as suggested by Carr & 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 & Black (1980) was employed. Relevant meiotic and mitotic stages were microphotographed.

Results

Conventional staining

Spermatogonial metaphase possesses 25 elements, out of these, 24 are autosomes and the X chromosome is the 2nd smallest element after the m chromosomes. A pair of m chromosomes and the largest chromosomes are clearly distinct (Fig. 1a). In the diakinesis, 13 elements are visible. Out

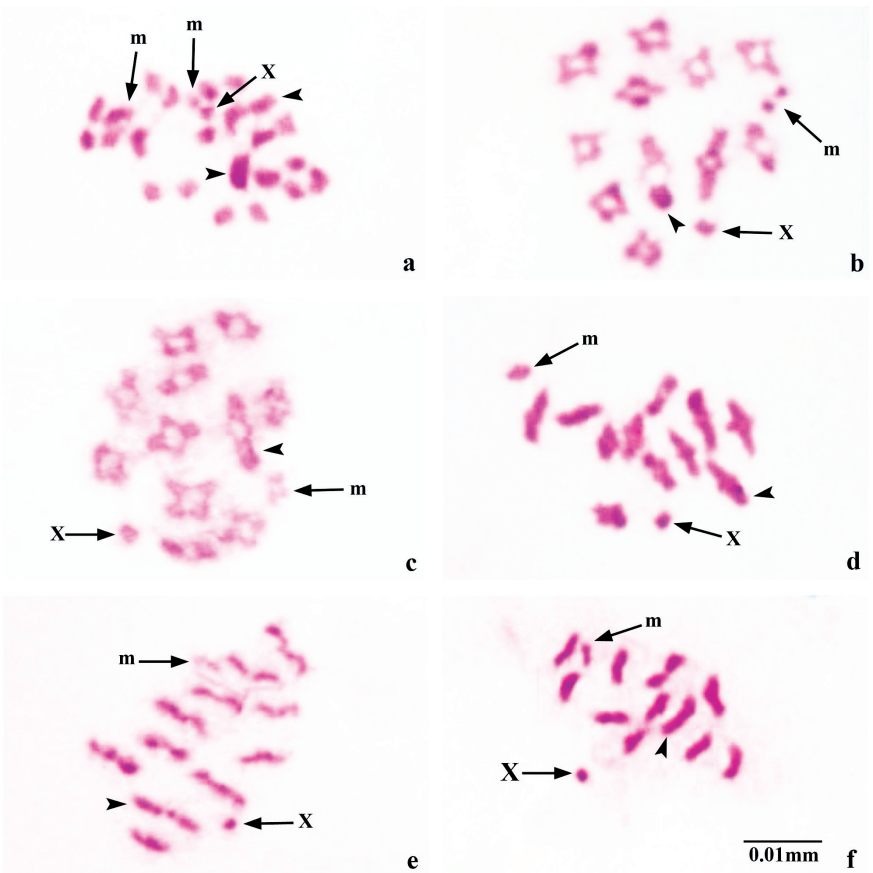

Figure 1. Spermatogonial and primary spermatocyte chromosomes of *Gynacanthaeschna sikkima* from India. a – spermatogonial metaphase; b, c – diakinesis; d – metaphase I; e – prophase II; f – metaphase II. Arrowhead shows largest autosomal bivalent. X and m are marked with arrows.

of these, 12 are autosomal bivalents while X univalent lies away from the centre. All the autosomal bivalents, including the m bivalent, show single chiasma while the largest autosomal bivalent possesses two interstitial and terminal chiasmata (Figs 1b, c). During metaphase I, the bivalents are small because of the condensation and terminalization of chiasmata and the X chromosome lies at the periphery (Fig. 1d). During Prophase II 'ε' shaped chromosomes, which are characteristic of odonates, are present. Due to the small size and light stain, respectively, X and m chromosomes are distinct (Fig. 1e). In the metaphase II, the chromosomes are half the size of metaphase I chromosomes, while X and m are clearly distinguishable (Fig. 1f).

C- banding

During diakinesis, all the autosomal bivalents, except the m bivalent, possess terminal C-bands. The largest autosomal bivalent shows two interstitial and terminal C-bands while the X chromosome has a large C-band on one side only (Figs 2a, b, c). In the metaphase II, all the autosomal bivalents, including the largest bivalent, have terminal C-bands, while m is C-negative and X is C-positive (Fig. 2d).

Silver nitrate staining

During the diffuse stage, the darkly stained nucleolus and X chromosome are clearly visible (Fig. 2e). During diakinesis, the X chromosome has a large interstitial NOR band, while nine autosomal bivalents, including the m bivalent, have terminal NOR band on one side only (Fig. 2f).

Discussion

In the Aeshnidae, 2n = 27 (26+X0) is considered as the modal number of chromosomes (Lefevre & McGill 1908; Makalovskaja 1940; Oksala 1943, 1944; Omura 1957; Seshachar & Bagga 1962; Cumming 1964; Cruden 1968; Kiauta 1968, 1970, 1972, 1975, 1979; Hung 1971; Tyagi 1982; Tyagi & Sangal 1982; Thomas & Prasad 1986; Mola & Papeschi 1994; Mola 1995; Walia & Sandhu 1999; Walia 2007). During the course of karyotypic evolution in the family, the chromosome number varies from 2n = 14–27 and sex determination from X0 to neo-XY (Cumming 1964; Seshachar & Bagga 1962; Kiauta 1967, 1971, 1975; Ferreira et al. 1979;

Tyagi 1978, 1982, 1986; Kiauta & Kiauta 1982; Mola & Papeschi 1994; Sandhu & Malhotra 1994; Mola 1995; Walia & Sandhu 1999; Walia 2007). Reduction in chromosome number is due to the fusions between autosomes, while the neo-XY sex determining mechanism originated by fusion of autosome with the sex chromosome.

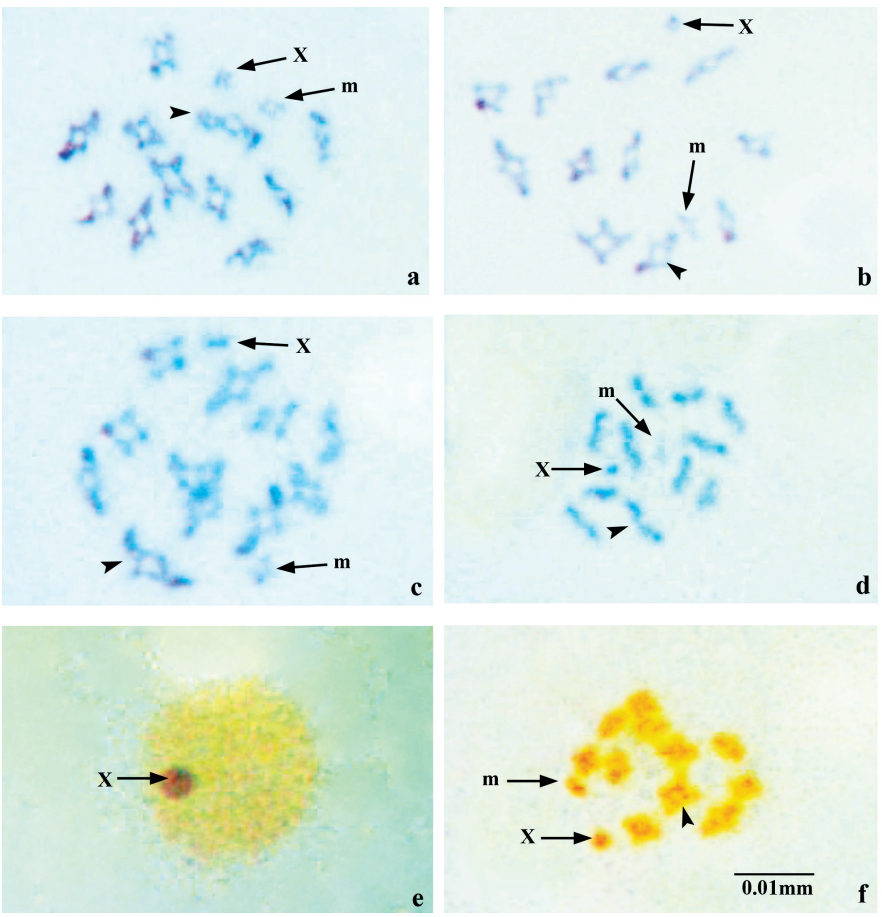

Figure 2. Spermatogonial and primary spermatocyte chromosomes of *Gynacanthaeschna sikkima* from India. a–d – C-banding: a, b, c – diakinesis; d – metaphase II. e, f – silver nitrate staining: e – diffuse stage; f – diakinesis. Arrowhead shows largest autosomal bivalent. X and m are marked with arrows.

In the present study, chromosomal investigations on *Gynacanthaeschna sikkima* by conventional staining, C-banding, and silver nitrate staining have been carried out for the first time. This is the only known species of the genus, possessing a 2n(♂) = 25m with X0(♂)/XX(♀) type sex determination. The reduction in chromosome number from the modal number is due to the fusion of a pair of autosomes which is confirmed by the presence of two large chromosomes in the spermatogonial metaphase. During diakinesis, the largest bivalent shows two interstitial and terminal chiasmata, whereas a single chiasma per bivalent is present in other odonate chromosomes.

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